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ADVANCES IN PEST CONTROL RESEARCH

Volume IV

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ADVANCES IN PEST CONTROL RESEARCH

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Introduction to the Series

Pests—organisms that compete with man for his food supply, damage his possessions, and attack his person—are of vital concern to all mankind. Included in this category are some thousands of economically destructive species of plants and animals, variously classified as viruses, bacteria, fungi, weeds, protozoa, nematodes and other helminths, molluscs, insects and other arthropods, fish, birds, and rodents. The accumulated attacks of all of these pest organisms cost the human race incalculable damage in direct loss of vital foodstuffs, in enormous expenditures for pest control activities, and in pestilence and disability.

Pest control in its broadest sense in a continuous warfare against man's competitors, carried out on countless fronts with operations ranging in scope from those of the housewife and home gardener to the global efforts of the Food and Agriculture Organization and the World Health Organization of the United Nations.

Throughout the centuries, pests have demonstrated amazing plasticity in their abilities to circumvent man's best efforts to control them, and presently it is abundantly clear that research in pest control not only is essential to ensure human enjoyment of the fruits of the earth but also is capable of yielding unsuspected dividends in terms of an expanding population with abundance for all.

Pest control research currently occupies the attention of a significant portion of the world's scientific manpower, at work in university, governmental, and industrial laboratories, and in field experimentation the world over; increasing the basic knowledge of our animal and plant competitors and developing and applying more suitable and effective measures for their control. Although these research endeavors are often carried out under the various classical disciplines of plant pathology, entomology, economic botany and zoology, it is apparent that they have much in common. This has been recognized the the employment of such terms as plant protection or Pflanzenschutz research. Furthermore, progress in pest control research is directly dependent upon the ancillary disciplines

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of chemistry, physics, ecology, and pharmacology. It is therefore to this broad field of endeavor that *Advances in Pest Control Research* is addressed.

The subject matter will be selected from recent significant research trends related to all phases of pest control, with emphasis on the fundamental aspects. As in other "Advances," the writers will be specialists in the various fields who will be urged to contribute not only comprehensive reviews but also critical evaluations of new concepts and developments. It is hoped that by the encouragement of the presentation of personal viewpoints, *Advances in Pest Control Research* will become a stimulating vehicle for the fostering of new lines of research as well as a continuing reference work for the research worker, the teacher, and the student. Advice and counsel leading to the best approaches to these objectives are earnestly solicited.

R. L. METCALF

Riverside, California
June, 1957

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SOME FUNDAMENTAL ASPECTS OF APPLIED INSECT PATHOLOGY

By IRVIN M. HALL, *Riverside, California*

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I. Introduction

The field of insect pathology has broadened rapidly during recent years, and there has been a great increase in our knowledge of microbial diseases of insects. In the wake of this steady growth, a widespread appreciation of the study of diseases of insects has evolved, and there has developed a sweeping interest in the use of microorganisms for the control of insect pests.

The last thorough work on the subject of insect pathology was by Steinhaus (30). Since that time the effects of disease on insect populations (32) and the historical aspects (34) have been covered by

Steinhaus, and recently several excellent reviews of insect pathology have appeared (Bergold, 7; Steinhaus, 37; and Tanada, 41). Bucher (9) also has presented a review of the utilization of disease to control insects. In addition, a compilation of references on the "artificial" control of insects by means of entomogenous fungi and a discussion of the use of fungus diseases in the biological control of insects have been prepared by Baird (3,4). With such thorough coverage of the literature available to the reader, there will be no effort to develop this paper into an additional review of recent advances in the use of microorganisms for the control of insects. Instead, an attempt will be made to furnish a brief but concise coverage of the fundamental aspects of applied insect pathology, with references where necessary, to serve the needs of the entomologist who is making his first approach to the use of microorganisms as a means of insect control.

II. Terminology

In order to deal with the various phases of applied insect pathology, the economic entomologist should have an understanding of the rudiments of infection and epizootiology. Although it will be impossible to go into the subject at length in this paper, the various important definitions will be covered in order that at least the basic terminology used in the microbial control of insects will be understood. For a more thorough treatment of this topic the reader is referred to Steinhaus (30,32).

Infection is a biological relationship in which a microorganism invades, grows, and reproduces in the tissues or body fluids of the host. The resultant effect is disease. An *infectious disease* is a disease caused by the presence of a living organism. It is considered to be contagious when naturally transmissible from one host to another by direct contact. An *intoxication* is due to the presence of symptoms caused by the activity of microorganisms which may not necessarily be present in the afflicted individual.

The term *virulence* refers to the ability of a microorganism to invade and injure the tissues or body of its host. It is generally used to indicate an attribute of the microorganism, as distinguished from the *resistance* or *susceptibility* of the host. *Pathogenicity* may be considered to be synonymous with virulence since a *pathogen* is a microorganism that can cause an infection or disease in a particular host.

True pathogens are capable of invading under normal conditions of host resistance and rarely live in close association with the insect without producing disease. On the other hand, occasional pathogens, or secondary invaders, are those microorganisms that live in more or less constant association with their host and which invade susceptible tissues and cause disease when the host has become weakened by some other factor in the environment.

The *portal of entry* is the point at which the microorganism enters the body of its host. In general, the portal of entry will vary according to the group of microorganisms concerned. Most bacteria, viruses, protozoa, and nematodes enter the insect body by way of the mouth and intestinal tract. On the other hand, most fungi enter the body cavity by penetrating the integument or body wall of the host. The *incubation period* of an infection or disease is the time elapsing between the entrance of the microorganisms into the insect body and the development of symptoms. When the pathogens multiply in the blood and tissues throughout the body cavity of the insect and cause harmful reactions, the condition is known as a *septicemia.* A *toxemia* is a condition produced by the dissemination of toxins or other poisonous substances in the blood.

Epizootiology is the science that deals with infectious diseases as they occur in groups of animals. The word has essentially the same meaning in regard to other animals as has the term "epidemiology" in reference to human beings. The term *morbidity* refers to sickness or disease, while *mortality* refers to death. *Incidence* of a disease relates to the degree of its occurrence in a particular population. The *prevalence* of a disease is the product of its incidence and duration. An *epizootic* disease is a disease (or phase thereof) of high morbidity and one that is only occasionally present in recognizable form. An *enzootic* disease is one that has a low incidence but is constantly present in a population.

III. Mode of Action of Insect Pathogens

Before attempting to utilize microorganisms in the control of insect pests, the entomologist should be familiar with the modes of action of the groupings of entomogenous species against their hosts. In general, the entomogenous microorganisms may be divided into two groups according to the natural portal of entry into their hosts.

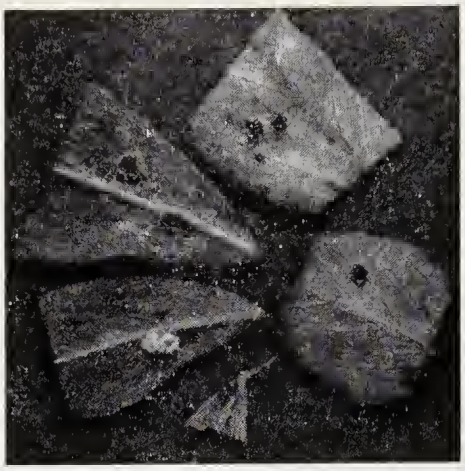

Fig. 1. Spotted alfalfa aphids infected with the fungus *Entomophthora exitialis* Hall & Dunn. (Original photograph by K. Middleham)

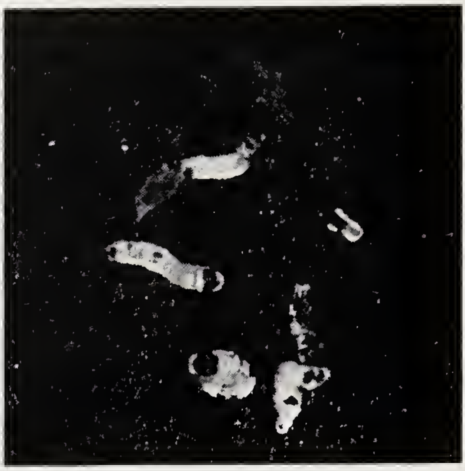

Fig. 2. Mummified larvae of the lawn moth, *Crambus bonifatellus*, killed by a strain of *Beauveria bassiana* from the European corn borer. (Photograph by K. Hughes; from Hall, 20a)

The first group, infecting the host through the integument, includes the large number of entomogenous fungi (Figs. 1 and 2). In essence, this group could be considered to be similar in activity to the contact chemical insecticides, since ingestion is not required for infection. Following penetration of the host integument the organism develops rapidly, and the body contents of the insect are consumed with resultant death to the host individual. It is of interest that many of the fungi have special methods for formation and dispersion of their transitory stages in order to enhance their spread from host to host. Some fungus species are particularly well developed in this regard, with special holdfasts to secure the dead host in an elevated location and externally formed spore stages for easy dispersal by environmental factors or by contact with healthy hosts.

The second group, which includes the bacteria, viruses, protozoa, rickettsiae, and many nematodes, may be considered to be similar to the stomach poison type of chemical insecticides, since the organisms must be ingested in order to cause infection (Figs. 3 and 4). Following ingestion by a susceptible insect, the organisms penetrate the wall of the alimentary tract and are carried by the body fluids to the cells or tissues where they develop. The bacteria generally

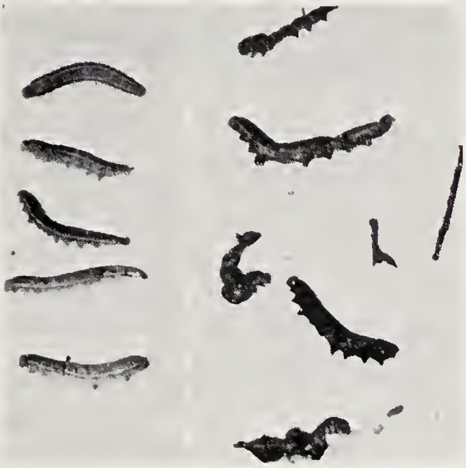

Fig. 3. Alfalfa caterpillars infected with polyhedrosis virus. *Left:* larvae healthy; *right:* larvae diseased. (Original photograph by K. Hughes)

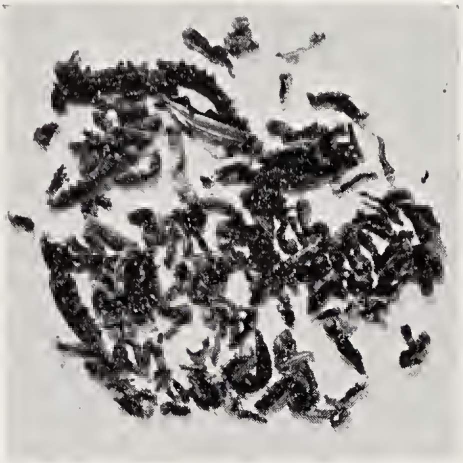

Fig. 4. Alfalfa caterpillars infected with the crystal-bearing bacterium *Bacillus thuringiensis.* (Original photograph by K. Hughes)

cause a septicemic condition by developing profusely throughout the body fluids and tissues of the host. In addition, the crystal-bearing species of spore-forming bacteria are noted for their toxicity to susceptible hosts. On the other hand, the viruses, protozoa, and rickettsiae are more specific in their sites of development and grow only in selected tissues within the insect body.

IV. Production of Entomogenous Microorganisms

One of the main obstacles to field experimentation with microbial materials up to the present time has been the difficulty of producing pathogens in quantities large enough for other than very small field tests. However, it appears that this bottleneck at last is being broken. Great interest is being shown by various commercial concerns in the production of microbial products for use against insect pests, and it is quite possible that within a reasonable length of time an ever-growing list of entomogenous microorganisms will be made available to applied entomologists for laboratory and field screening programs. The commercially produced insect pathogens probably will be limited initially to those microorganisms that can be produced readily on artificial media. However, if commercial interest continues and microbial control methods are accepted for general use, some of the effective but more difficult to produce microorganisms should become available for field use.

The entomologist desiring to test organisms that are not commercially available will have to use other means to obtain supplies of microbial materials. These methods vary widely according to the organisms being produced, and many obstacles have to be surmounted as production techniques are developed. In order to give an idea of the techniques that have been developed for the rearing of insect pathogens, some of the classic methods will be discussed.

Thompson and Steinhaus (42) used two methods to build up a supply of polyhedrosis virus material for use against the alfalfa caterpillar, *Colias philodice eurytheme* Bdv. Because of the obligate nature of the virus parasitism, both methods dealt with production in living host insects. The first technique, which is of value if an initial supply of virus is desired early in the season when natural epizootics are not likely to occur, was to infect and rear, in the laboratory, healthy larvae collected in the field. The second method was to collect larvae dead and dying of the disease in the field.

By loading rearing trays with field-collected larvae (Fig. 5), Thompson and Steinhaus were able to produce up to 1 liter of virus-killed insects per tray in 7 days. A total of 4 man-hours was required to feed and maintain the larvae during the incubation period and to

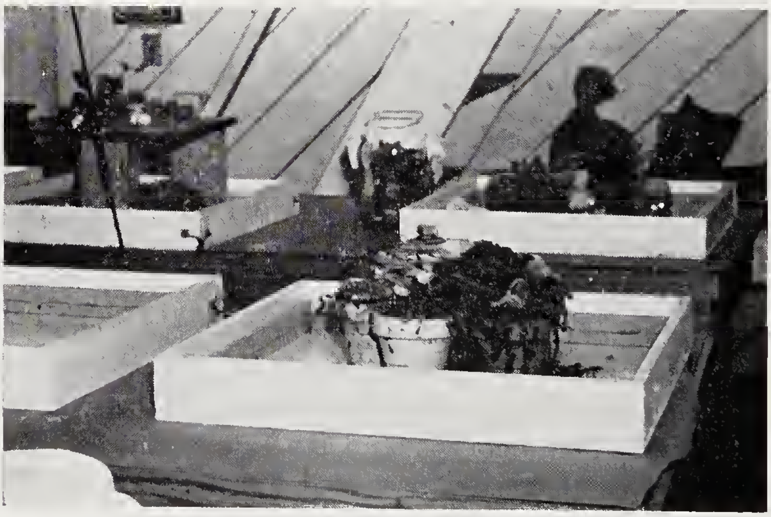

Fig. 5. Trays with electric barriers for the production of infective material. (Original photograph by K. Hughes)

remove the virus-killed larvae for placement in storage containers. They determined that one man, by starting a new culture in a rearing tray each day, could produce a liter of virus material daily once production was underway. Although this method requires a field source of caterpillars, they state that it would be possible to rear the larvae entirely by insectary methods. Such a program was utilized by McEwen and Hervey (28) for the production of a limited quantity of virus material from the cabbage looper, *Trichoplusia ni* (Hbn.), an insect that is not easily collectable in large numbers from most of its host plants other than by hand picking.

Following the initial accumulation of virus, according to Thompson and Steinhaus (42), the least expensive way of increasing the supply is to spray a field containing a high population of caterpillars. By

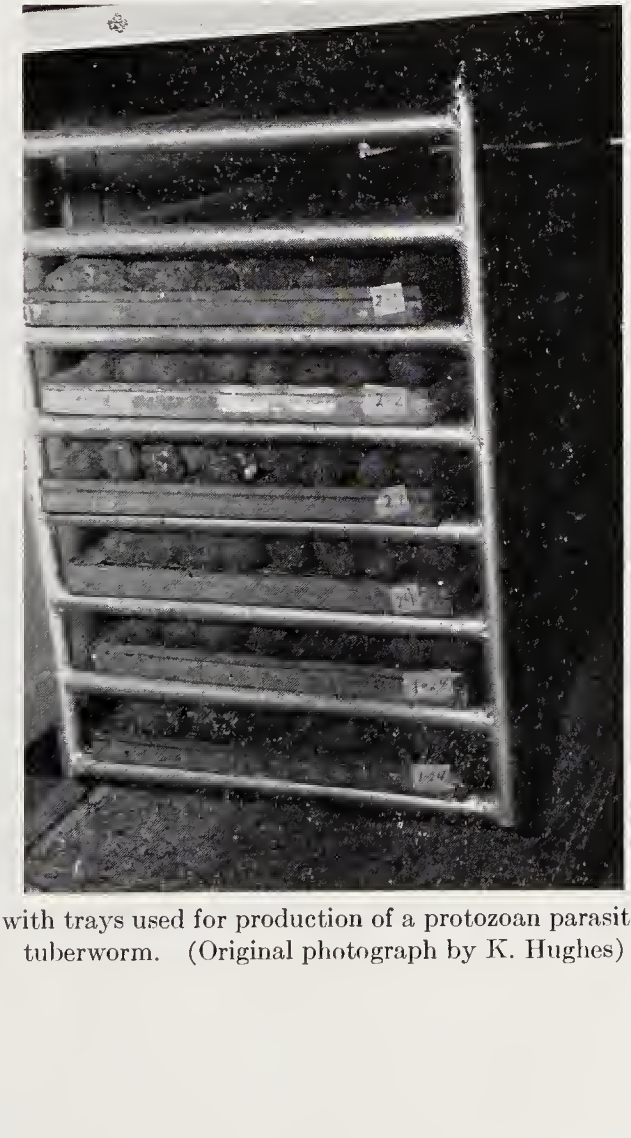

Fig. 6. Rack with trays used for production of a protozoan parasite in the potato tuberworm. (Original photograph by K. Hughes)

gathering the infected larvae on the day before they can be expected to die of the disease, depending upon the ease of collection, large quantities of the virus may be obtained. Additional material can be gathered as needed by collecting diseased larvae in virus-treated fields at the proper time after application. The virus can be stored in almost any manner and will be ready for use the following year when populations of the caterpillar first appear.

The entomogenous protozoa are obligate parasites that are known to grow only in living hosts. There is little chance that large quantities of infected larvae can be collected in the field. Therefore, if a protozoan parasite is to be tested in the field, it probably will be necessary to produce quantities of the resistant spore stage in the laboratory by a method similar to that used for virus production (Fig. 6).

Many of the fungi pathogenic to insects are known to grow readily on artificial media (Fig. 7), and techniques for their mass culture in

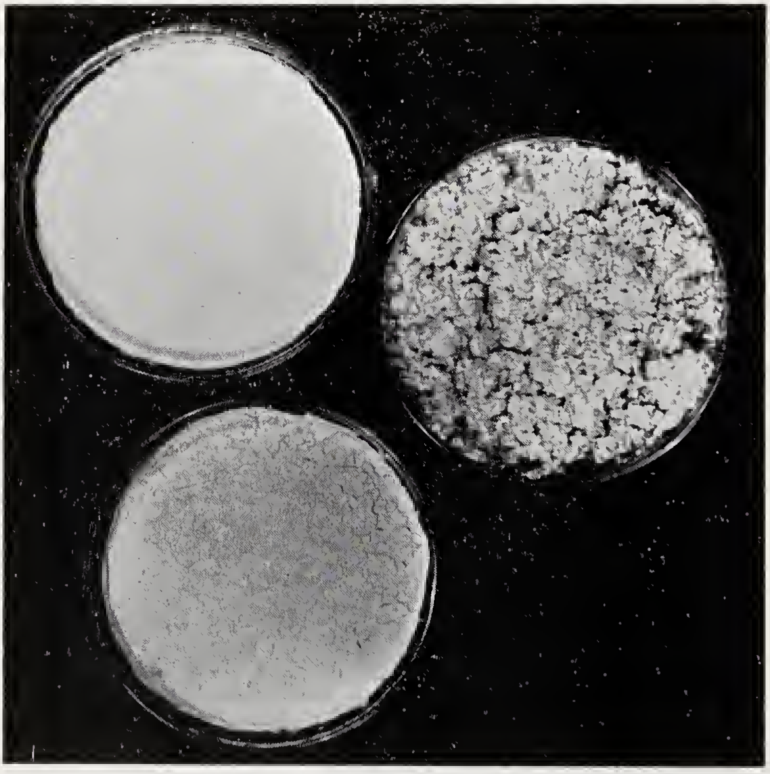

Fig. 7. Colonies of *Beauveria bassiana* growing on nutrient agar, oatmeal, and wheat bran. (Photograph by K. Hughes; from Hall, 20a)

the laboratory may be developed without too much difficulty. The fungus *Beauveria bassiana* (Bals.) Vuill. has been produced in quantity by several workers. Bartlett and Lefebvre (6), in preparation for extensive field tests, produced large quantities of spore material on cornmeal mash in trays in a steam-sterilized culture cabinet similar in design to that described by Rorer (29). Dresner (11) tried various ways to produce *B. bassiana* spores in quantity. Unsuccessful methods included Erlenmeyer flask culture, cultivation on live vegetable matter, cultivation on whole seeds, and inoculation of large insects. Large-scale production of spores was accomplished with cultures on various bulk vegetable media in petri dishes. By culturing, collecting spores, and reculturing until the medium was completely utilized, Dresner produced spore material at the rate of 60 grams per pound of dried beans.

The production of entomogenous bacteria is not without its problems because of the variations in the nutritional and environmental requirements of the organisms. Many of these species do not form spores in their life cycle and may lose virulence if cultivated repeatedly on artificial media, but it is possible that they may be produced in quantity for immediate use in laboratory and field tests either by submerged culture techniques or by a method similar to the one described by Steinhaus (31) for the production of the crystal-bearing bacterium, *Bacillus thuringiensis* Berliner. Appreciable quantities of that organism were grown on the surface of nutrient agar in 6 liter Povitsky bottles (Fig. 8). The medium in each bottle was inoculated

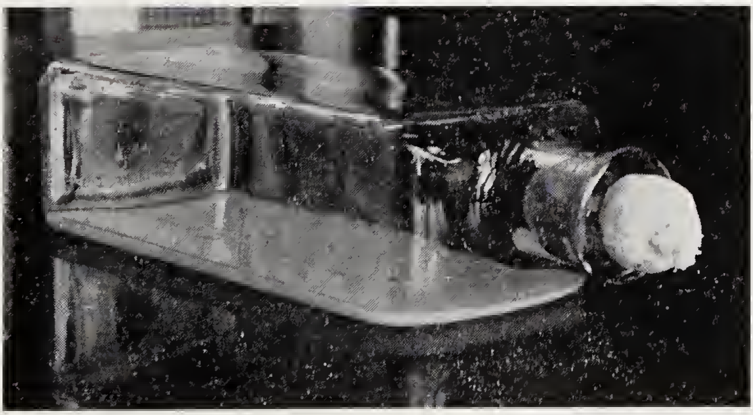

Fig. 8. Povitsky bottle with growth of the crystal-bearing bacterium *Bacillus thuringiensis*. (Original photograph by K. Hughes)

with a diluted culture of the bacillus, and the bottles were incubated at about 23°C. for about 7 days. Upon completion of the incubation period, the spores were harvested by washing the growth off the agar. The resulting suspension, which was cleaned by centrifugation and filtered through cheesecloth to remove stray bits of agar, was poured into flat enameled pans and allowed to dry. The brittle deposit of spores and crystals was then scraped from the pans and ground into a powder with a mortar and pestle.

On occasion it may be necessary to grow an entomogenous bacterium in a susceptible living host in order to produce quantities of the resistive stage. Such a method was developed by Dutky (15) for the production of spores of *Bacillus popilliae* Dutky, the cause of type A milky disease of the Japanese beetle, because the organism did not sporulate on artificial media. Although work is continuing on a method of artificial production, present day producers still cultivate the bacillus in the bodies of Japanese beetle grubs. Following completion of the incubation period, the infected grubs are crushed, and the resulting suspension is standardized by spore count. The suspension then is added to a carrier and the mixture is dried prior to compounding into a final preparation for use in the field.

It is doubtful that many entomologists will undertake the production of nematodes for field use, but it should be recognized that considerable work has been accomplished along this line. Few entomophilic nematodes have been reared apart from their hosts; however, this has been accomplished on occasion and efficient methods for production have been devised. Glaser (19) described the cultivation of *Neoaplectana glaseri* Steiner, parasite of the Japanese beetle, on a veal infusion–agar–yeast preparation. Glaser (20) also described a test tube method of growing nematodes in a yeast- and bacteria-free culture of animal tissue in a veal infusion–agar slant. A more inexpensive method was devised by McCoy and Glaser (27) in which nematodes can be produced in trays of fermented potato mash. Later, before the extensive studies of the nematode were discontinued, a similar but more productive way, using an infusion veal pulp, was developed by McCoy and Girth (26).

It is possible the living hosts may be used for the production of quantities of resistive stages of certain nematodes. Dutky and Hough (17) and Dutky (16) report the successful mass culture of a nematode in larvae of the greater wax moth, *Galleria mellonella* (L.). They obtained yields of up to 150,000 infective stage nematodes per infected wax moth larva. The nematodes were maintained in the bodies of dead hosts or recovered by means of a special nematode trap and stored in a refrigerated or aerated jar of pure water.

V. Determination of Insect Susceptibility

The effectiveness of a given pathogen against certain insect pests can be evaluated in field tests by the careful analysis of results, along with observations and microscopic examinations of dead insects from the test plots. However, many insect pathologists prefer to precede these efforts, if possible, by the determination of susceptibility in laboratory experiments. If laboratory evaluations are undertaken, it is important that the tests be conducted against the same stage (instar) of insect that would be subject to control in the field programs. On occasion, some workers conduct these tests by intrahemocoelic inoculation, thereby assuring entry of the organism directly into the body cavity. On the other hand, since even the least pathogenic microorganism may grow profusely and cause mortality in any host when provided with an abnormal method of entry, most insect pathologists choose to determine the effectiveness of an organism using only the natural routes of entry that would be required for successful infection in the field.

Susceptibility determinations of the entomogenous fungi that can be expected to enter the bodies of their hosts through the integument may be accomplished by direct application of the transitory spore stages of the pathogens to the external surfaces of the test insects. Since the fungi generally are quite responsive to temperature and humidity conditions, it is best that these factors be controlled in order to give the pathogen the best possible chance for successful penetration and resultant infection. As in all tests of this nature, suitable control groups of untreated hosts should be reared simultaneously to ascertain the condition of the stock insects.

Organisms such as the bacteria, protozoa, and nematodes, which normally enter their host through the alimentary tract after being ingested, should be evaluated initially in oral feeding tests, although topical applications may be tried in subsequent experiments. The methods for conducting feeding tests vary greatly according to the desire of the entomologist and the needs of the particular test being run. Some individuals prefer to run comprehensive feeding tests for the determination of median lethal dose (LD_{50}) or time (LT_{50}), while others choose to limit their initial studies to the gross determination of susceptibility. In the latter situation, suitable food materials are contaminated with a pathogen by dusting, spraying, or dipping and then fed to a group of test insects. The simultaneous rearing of untreated control insects is a necessity to check the condition of the test stock. This is particularly important if the test insects are from a field source. Adequate control of the test environment should be maintained, especially for determination of the incubation period of the disease, although temperature and humidity requirements are not as critical as with the fungi that must germinate outside the host prior to penetration of the integument.

Bucher (9) considers the LD_{50} as the best quantitative measure of the pathogenicity of an organism for a given host. However, he states that the LD_{50} of a pathogen for a host may fluctuate rather widely because strains of the pathogen vary in virulence and because host populations display variable resistance (insects may have different degrees of resistance to infection at different ages and under different conditions of stress).

It will be necessary to run infectivity tests for periods long enough to obtain maximum mortality figures, although the time will vary greatly according to the microorganism being tested, the susceptibility of the host insect, and the conditions of the test. In general, the bacteria act rather quickly against at least moderately susceptible host insects, and if the test insects are given a single feeding of contaminated foliage, maximum mortality should be attained in less than 7 days. However, if a virus is tested against its normally specific host for the determination of the incubation period of the disease, the direct response of the microorganism to temperature may delay maximum mortality for some time if the temperature at which the test is run is low.

The concentration of a pathogen may have a direct effect on the degree of susceptibility of the host and the incubation period of the disease. Therefore, if possible, an accurate approximation of the concentration of the measurable infective stage (e.g., spores of spore-forming organisms, polyhedra of polyhedrosis viruses, etc.) should be made for each pathogen being tested. These concentrations can be determined in most instances in the laboratory with a simple counting chamber such as a hemacytometer.

A determination of mortality in each test can be made by periodic counting of either surviving or dead individuals in the treated and untreated groups of insects. After correction for mortality in the control population, the resultant percentage mortality will be an indication of the susceptibility of the host to the organism or of the effectiveness of the pathogen against the host. The results of any gross infectivity test will serve only to indicate the degree of susceptibility of a particular insect pest to a pathogen and should be used merely as a guide toward subsequent field tests with the organism. A determination of susceptibility in the laboratory cannot assure successful field use of a pathogen, since laboratory results often cannot be duplicated in the field where many uncontrollable factors influence the outcome of any biological action.

Field testing of pathogenic microorganisms without previous determinations of host susceptibility in the laboratory is not without its hazards because of the various uncontrolled factors influencing the insect population. However, when field tests are handled with care and augmented by the microscopic examination of the remains of the various insects recovered from the plots, evidence of the effect of the pathogen on the various insects on a crop can be obtained. To overcome the possible influence of side factors it is best to use high dosages of infective material in the initial tests. If they work, much lower dosages can be used in subsequent trials.

VI. Field Testing of Microorganisms

Over the years various means have been devised for the effective use of microorganisms for the control of insect pests. In general, taking into consideration dispersal characteristics, pathogenicity, speed of activity, and pest control requirements, they can be listed under two major methods of utilization. The first method involves direct introduction or colonization of the microorganisms, either singly or in combination (or coordination) with insect parasites, predators, or other entomogenous microorganisms, for the purpose of establishment of pathogens into an insect population for long-term reduction and possible eventual permanent control of the pest species. The second method involves direct application, singly or in combination with other microorganisms or compatible chemical insecticides, for quick control of the present economic pest population. As presented by Steinhaus (32) with regard to the dissemination of viruses already present in a population but applicable as well to other microorganisms, the application of a pathogen on an infested plant acts to lower the "threshold level" of the disease, thereby advancing the mortality peak of epizootics that occur naturally or initiating an epizootic that will result in a control that will be temporary in nature, much in the manner of chemical control measures.

In order to assure survival of the microorganisms until ingestion or contact by susceptible hosts, the pathogens should, when possible, be applied in their resistive stage: the bacteria, fungi, and protozoa as spores (or inclusions or combinations of inclusions and spores in the case of the crystal-bearing bacteria); the viruses, in polyhedra or capsules; and the nematodes, in the dauer stage. However, organisms which do not form resistant stages may be used if applied with a suitable protectant that will preserve the viability of the pathogen until ingestion or contact with a susceptible host.

Although application studies have been limited, entomogenous microorganisms have been successfully applied to crops with the various conventional ground and air equipment in general use in insect control programs. Because of the ease of preparation of suspensions from laboratory-produced or field-collected materials, considerable emphasis has been placed on the use of sprays by many workers, and the resistant stages of some organisms have shown the ability to withstand passage through nozzles at pressures of up to 1000 p.s.i. Less work has been done with dust applications over the years because of processing difficulties, but various crude dusts have been used experimentally to control a number of different insect pests (Fig. 9). It is evident that many pathogens are suitable for processing into dusts as well as into sprays, and it is likely that good dust formulations may be required in some experimental control programs to furnish the thorough coverage needed to reach the hidden portions of plants where the insect pests are feeding.

A. STANDARDIZATION FOR FIELD USE

A major problem for the insect pathologist or entomologist in the initiation of a field program to determine the effectiveness of a particular pathogen is the standardization of the infective material. Some form of standardization, of course, is a necessity if repetition is to be accomplished and is especially important if reliable dosage

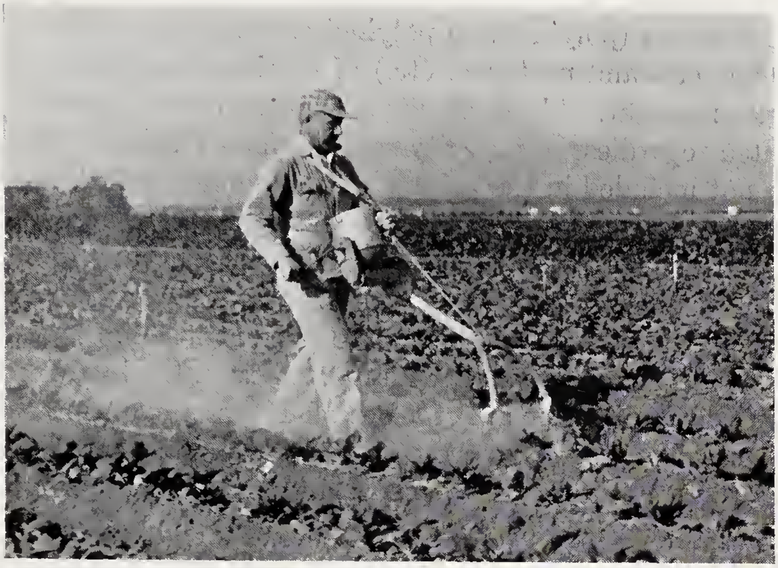

Fig. 9. Application of commercially produced *Bacillus thuringiensis* with hand-operated duster for control of the cabbage looper on crucifers. (Original photograph by J. Elmore)

levels of a particular entomogenous microorganism are to be developed for use in an insect control program. This problem is minimized to some extent with the use of a commercially produced material such as *B. thuringiensis,* since the manufacturer will furnish information on the concentration or potency of the particular lot or product and will offer suggestions to serve as a guide for proper usage in the field. On the other hand, difficulties will be encountered in attempts to standardize preparations of field-collected or laboratory-produced insect pathogens, and the facilities of a microbiology laboratory may be necessary for effective determinations of concentrations.

Early workers in applied insect pathology, for want of a better method, often used the infective contents of a specified number of diseased insects per unit of area (e.g., x larvae per acre) for their established level of dosage, assuming, no doubt, that the concentration of infective material per insect was uniform. This method still is in use in field studies with granuloses and polyhedroses viruses, and it does serve as a rough guide to the amount of infective material being applied in the tests. A much better, but still not precise, method employed by various workers in some determinations of concentration is to use a hemacytometer to ascertain the approximate number of resistive stages (polyhedra of polyhedroses viruses, spores of spore-forming bacteria and protozoa, etc.) of the insect pathogen per unit of volume or weight. These counts are open to error, since all stages are counted regardless of viability and the highly infective free virus particles not included in the polyhedra are ignored. Still, the hemacytometer count is a convenient and quick method of arriving at an approximate concentration of infective material for many of our most common entomogenous microorganisms.

A technique for the evaluation of the concentration of a granulosis virus infective material has been reported recently by Martignoni and Auer (25). With the aid of a Petroff-Hausser counting chamber and a phase microscope they were able to make an accurate determination of the concentrations of suspensions of a granulosis virus for use in a program of field tests.

Viable spore counts of spore-producing pathogens can be made by culturing known dilutions of spores on agar plates. However, there have been indications that the viable spore count of a crystal-bearing bacterium is not an accurate index to the insecticidal activity of the species, since the lethal effect of the organism is invested in the inclusions. With the spores and the products of their germination playing a secondary role, most workers dealing with crystal-bearing bacteria are beginning to rely upon a system of bioassay on certain uniformly susceptible laboratory insects in order to arrive at a rating of effectiveness.

B. DOSAGE DETERMINATIONS

In applied insect pathology the goal of the entomologist is to determine the minimum dosage at which a pathogen will give effective control of a particular insect pest. This can be arrived at with simple modification of application techniques in general use in the field evaluation of chemical insecticides. If a commercial material is being tested, the recommendations from the manufacturer or processor will serve as a starting point for the determination of the proper dosage level for each insect pest and crop. If recommendations are not available or an organism from other than a commercial source is being evaluated, the dosages of the initial field tests should be set at a high level and then adjusted up or down in future applications as dictated by the results.

It is difficult to suggest levels of concentrations of various pathogens for use in initial field tests because of all of the factors that ultimately must be considered. However, good starting materials for spray applications of the polyhedroses viruses may be suspensions containing 1, 5, and 10 million polyhedra per ml., applied at the proper gallonage for good plant coverage. For the spore-forming crystal-bearing bacteria a series of graduated dosages up to a level of 4.5 × 10^{13} spores per acre (that is, up to approximately 1 lb. of material of a concentration of 100 billion spores per gram or the equivalent toxicity per acre) applied as a dust or spray might be suitable. It is possible that similar levels may be usable as starting dosages for certain of the entomogenous fungi. Any starting recommendations for tests with protozoa or nematodes are difficult to make because of the lack of reports in the literature of successful control efforts. Nevertheless, it is possible that protozoa of the order Microsporidia might be applied initially at concentrations of up to 10 million spores per ml. and that nematode materials may be used at levels that will assure the application of a few thousand resistive stages to each plant.

C. TEST PLOTS AND EQUIPMENT

In general, the size of the test plots should be kept small in the initial tests to minimize the danger of crop damage (Fig. 10). Some

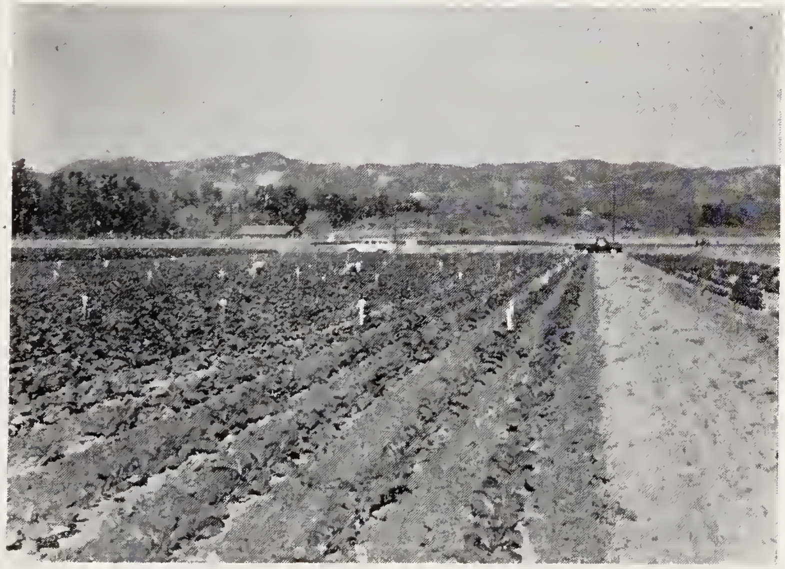

Fig. 10. Typical small row crop plot layout for testing preparations of pathogenic microorganisms. (Original photograph by J. Elmore)

modification may be necessary to prevent contamination of adjacent plots when dusts of almost any organism or the sprays of highly virulent pathogens such as the polyhedroses viruses are used. If such a danger exists, the drift can be minimized with the use of guard rows or larger plots.

In most successful field programs with microorganisms on truck and field crops the initial tests were made with the use of hand equipment because of the availability of only small amounts of test material. As the testing progressed and more material was produced or otherwise became available, in many instances ground power equipment (Figs. 11 and 12) was utilized, and on occasion the field studies were culminated with successful air applications of infective material (Fig. 13). Although most studies will advance in this fashion, it may be possible to bypass the small-scale preliminary applications in initiating tests with some of the materials that are available in quantity from commercial or other sources, and immediately run large-scale experiments with the same type of ground or air application equipment in general use for insect control in the area. In that event the value of the microbial material will be quickly determined for a particular insect control program.

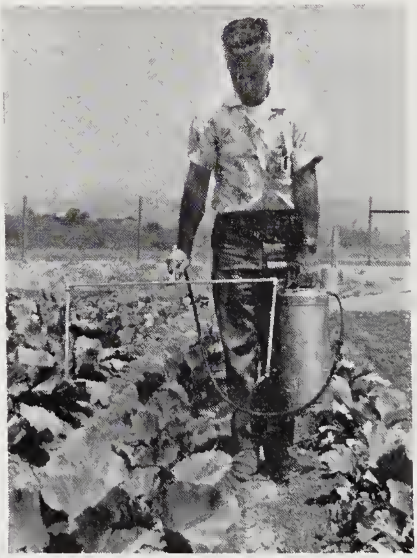

Fig. 11. Hand spray application of commercially produced *Bacillus thuringiensis* material against the cabbage looper on crucifers. (Original photograph by K. Middleham)

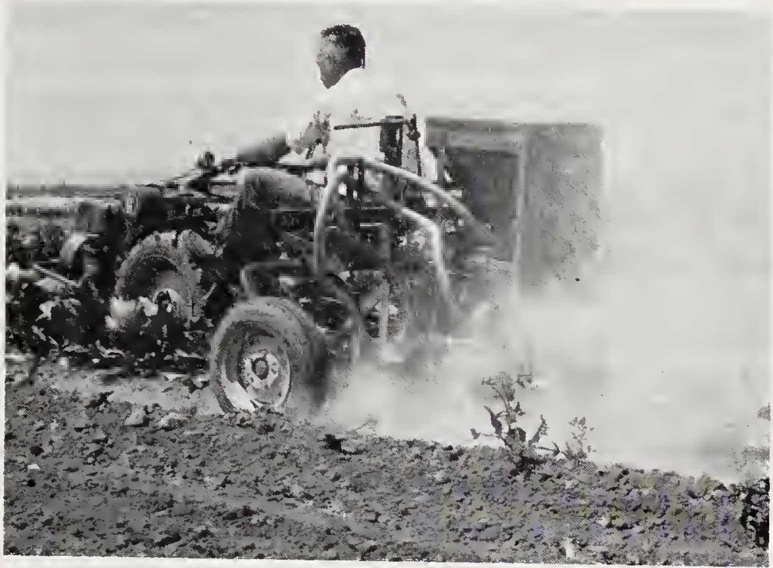

Fig. 12. Ground power dust application of commercially produced *Bacillus thuringiensis* material for control of the cabbage looper on crucifers. (Original photograph by K. Middleham)

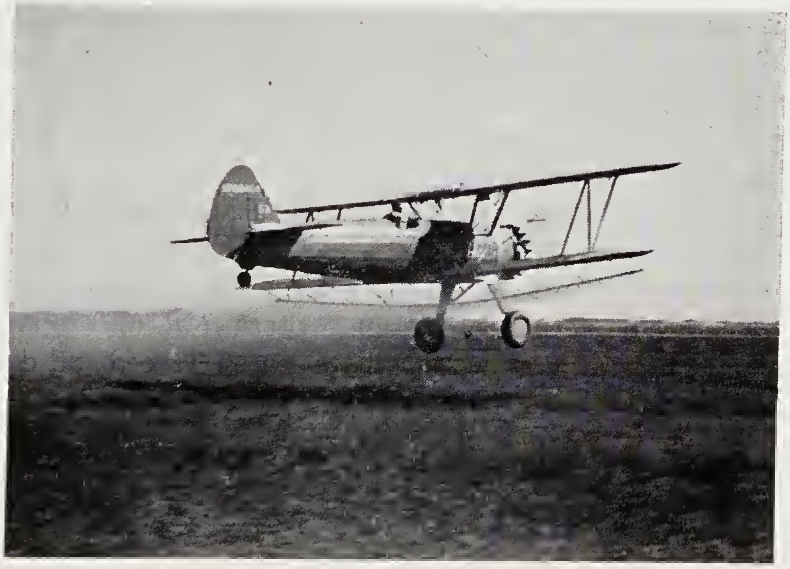

Fig. 13. Aerial spraying of polyhedrosis virus to control the alfalfa caterpillar. (Original photograph by R. Smith)

VII. Timing of Application of Insect Pathogen

In general, the timing of applications of sprays or dusts of entomogenous microorganisms is more critical than with the relatively quick-acting chemical insecticides, because of the time delay during the period of incubation from infection to the cessation of feeding of the insect host. This is an important factor to weigh when considering the use of pathogens in insect control programs on crops where even limited damage cannot be tolerated. Some microorganisms have very short incubation periods and can be compared favorably with many of the rapid-killing chemical insecticides. On the other hand, the slow action of other organisms may prohibit their use or force adjustments in the timing of application to allow for the delay.

Timing can be especially critical in the application of virus materials. In regard to the use of the polyhedrosis virus to control the alfalfa caterpillar, Thompson and Steinhaus (42) state that the timing of application may be so critical that the average grower or commercial applicator of insecticides may experience difficulty in determining when to use the material. Often, when the virus should be applied to allow for the incubation period delay, the caterpillars have not yet begun to cause noticeable damage, and the grower may not even be aware of their presence. Once damage begins to show in the field, it is usually too late to use the virus disease as a practical means of control, since the larvae will continue to feed for at least four days after application of the material. In fields with extremely high populations it may be necessary to apply the virus before the caterpillars hatch from the egg.

Timing, however, is not as critical with the utilization of the crystal-bearing bacteria such as *B. thuringiensis*, which compare favorably with chemical insecticides by causing death to their susceptible hosts as quickly as one day after application. Stern *et al.* (39) report that fourth and fifth instar alfalfa caterpillars ceased to feed and dropped from the foliage within 24 hours after the application of low dosages of a commercially produced *B. thuringiensis* material. The speed of the action of the bacillus was, in all respects, equal to that of the best chemical insecticide recommended for use against the alfalfa caterpillar.

VIII. Plant Coverage

An important factor in the application of microorganisms is the matter of proper plant coverage in order to obtain satisfactory kill of the susceptible insects. Since many pathogens have a mode of action essentially like a stomach poison, whereby the susceptible host must ingest enough of the infective stages to sicken and die quickly, it is evident that complete or good coverage of the feeding areas on the plant is essential in order to attain a significant reduction of the pest population within a reasonable length of time. Thorough coverage often is difficult to attain, expecially on low spread-out plants where the insects may be feeding on the under surfaces of the leaves or in otherwise protected areas. Fortunately, many of the pathogens are suitable for processing into various types of formulations, thus broadening the opportunities for experimentation with different application techniques.

IX. Compatibility of Microorganisms with Adjuvants and Chemical Insecticides

Although knowledge of the effects of additives in spray or dust formulations of insect pathogens is limited, various standard wetting and sticking agents, such as blood albumin spreader, skim milk powder, and flour, have been used successfully in the past in combination with microbial materials. Recently, some of the new surface-active agents have been utilized to aid in the application of various microorganisms, and commercially produced *B. thuringiensis* wettable powder formulations with built-in wetting agents have made their appearance. All in all, it appears that most of the adjuvants should have no deleterious effect on the various microbial materials and the proper use of such additives when needed to aid in better application and retention on the foliage should enhance the effectiveness of the organisms.

Very little information is available on the effect of various agricultural chemicals on entomogenous microorganisms. However, there is some evidence that many of these materials may not interfere with the activities of certain pathogens, either by being compatible or because they are used when the organisms are not present in the field. In tests with a polyhedrosis virus against the cabbage looper, McEwen and Hervey (28) found no indication that the addition of TEPP affected the activity of the virus in any way. A similar observation was made by Andres and Hall (1) when a mixture of Phosdrin* and virus was used in tests against the cabbage looper on lettuce. According to Dutky (16), the ensheathed larvae of the nematode isolated from codling moth larvae are quite resistant to chemicals. Hall and Dunn (24) were able to show in laboratory studies that certain species of entomophthoraceous fungi display varied reactions to several insecticides and fungicides. The vegetative stages of most species were more susceptible, while the resting spores of one species were most resistant but were delayed in germination when exposed to insecticides. They did not consider that the fungi would suffer seriously from insecticidal treatments of alfalfa fields where they occurred, since the organisms could be in the inactive resting spore stage when chemicals were in general use.

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* Registered trademark Shell Chemical Corp., New York, N. Y.

X. Effects of Physical Environmental Factors on Pathogen and Host

It is widely recognized that temperature and humidity are the principal physical factors that affect the activity of entomogenous microorganisms. According to Tanada (41) these factors may affect the pathogen (its survival and ability to infect), the host (its susceptibility or resistance, including activation of latent infections), and the progress of infection within the host. The roles that these factors play vary according to the type of organism. Pathogens such as the fungi that enter their host through the integument following external germination are quite responsive to humidity conditions, and lack of adequate moisture can inhibit infection even though the host is highly susceptible. Dresner (11) believes that entomogenous fungi can be depended upon to control insect pest infestations in areas of frequent rainfall or high humidity, such as the Atlantic Coast of the United States, but areas without this weather pattern should not expect insect control with the use of fungi. In general this is correct, but the surprising ability of pathogenic fungi to kill the spotted alfalfa aphid in irrigated fields in many otherwise dry areas of the United States and Mexico in recent years suggests the importance of the microclimate surrounding the organism and its insect host. On the other hand, the microbial agents of the stomach poison type, such as the viruses, the bacteria, and the protozoa, function irrespective of humidity conditions in the field, since they remain in their resistive stages until ingested, whereupon the body fluids and tissues of the susceptible hosts offer an ideal environment for development.

Temperature can play a critical role in the success or failure of microorganisms to control insect pests. Although the actions of the entomogenous bacteria do not appear to be influenced to any great extent by such variations, it is well known that temperature levels have a definite effect upon the incubation period of virus infections. In this regard, Thompson and Steinhaus (42) report that within the temperature range in which the alfalfa caterpillar is active, susceptibility to infection by the virus is independent of the temperature, but the length of time required for mortality decreases as the temperature rises. This is an important point to consider when the utilization of a virus is being contemplated, since relatively low temperatures during the post-treatment period could result in poor control of the insect pest.

XI. Pathogen Persistence in the Field—Residual Activity

Although there have been only a few determinations of the persistence of artificially introduced pathogens in the field, there are a number of reports of the survival of entomogenous viruses, bacteria, and fungi for long periods in the soil, in infected individuals, in the bodies of dead insects, or directly on the foliage. The presence of such characteristics for survival as can assure infection of subsequent host generations may mean the difference between success or failure in attempts to colonize microorganisms or in the experimental application of pathogens for the control of insects which have more than one generation per crop. However, the ability to survive is not considered to be a factor affecting the efficiency of the crystal-bearing bacteria, such as *B. thuringiensis*, since the insecticidal properties of these pathogens are invested in the inclusions and the organisms are not expected to perpetuate themselves in the field. In this case, as with chemical insecticides, the residual activity of the toxic stages applied to the foliage is of prime importance.

The residual activity is not important when a highly pathogenic material is applied for the control of an insect that develops only one generation during the crop-growing period (i.e., one alfalfa caterpillar generation per cutting), since the pest population, once reduced, does not build up again during the same cycle of plant growth. However, on many crops the residual effect of a pathogenic material applied to the foliage can be an important factor affecting its use, since continuous overlapping generations of the insect pest may occur, and the necessity for retreatment then will depend upon the lasting effect of the pathogen. Since the remains of virus-killed larvae usually stay on the foliage unless dislodged by physical agents, the viruses should have a lengthy residual activity limited only by weathering action and the rate of growth of the plant. On the other hand, the insects killed by protozoa and bacteria generally fall intact from the foliage, and since no buildup of infective stages can occur on the plants, the lasting effect of the pathogens should be expected to be much reduced. In their report of tests with commercially produced *B. thuringiensis* against insects on crucifers, Hall and Andres (21) state that the bacillus, without the aid of special sticking agents, displayed a residual effect of only 5–10 days against continually developing populations of cabbage loopers and imported cabbageworms on rapidly growing plants. It is apparent that the period of effectiveness of a pathogen will be dependent not only upon the host-parasite relationship, but also on the plant growth characteristics and the effect of physical factors in the environment.

XII. Insect Resistance to Microorganisms

The widespread development of resistance to chemical insecticides has not only created increased interest in the use of insect pathogens, but has also caused some concern that similar problems of resistance to entomogenous microorganisms may appear. It is difficult at this time to make an authoritative declaration concerning the possibilities of the development of resistance to insect pathogens, since field observations and testing have been so limited in scope and so little is known of the natural or acquired resistance of insects to disease. However, there is no positive evidence to date that insects have become resistant to infection from applied microorganisms or their byproducts. It is realized that resistance is difficult to evaluate and probably could not be readily recognized as such if it did develop in any area where only limited microbial testing was under way. Therefore, full field evaluation of resistance to an insect pathogen will have to await wide-scale testing and broad commercial use of microbial materials. Since most applied entomologists are cognizant of insect resistance to insecticides, any resistance developing to treatments with a widely tested organism such as *B. thuringiensis* should be detected quickly.

XIII. Selectivity of Insect Pathogens

The variations in the activity of entomogenous microorganisms against different insects is quite marked. Some organisms essentially are specific in their activity while others show varying degrees of pathogenicity for many species of insect pests. In the former category are the viruses which, in general, are considered to have specific hosts, while the latter group contains large numbers of organisms, including certain of the fungi, the entomophilic nematodes, and the crystal-bearing bacteria.

The ease with which an entomogenous microorganism may be worked into a control program will vary with the particular pest program. If it is necessary to treat for only one insect pest, with everything else being favorable, it is quite possible that applications of a single pathogen may bring about and maintain effective control. On the other hand, if several pest populations are present on the same crop, it is likely that even a nonspecific pathogen, like *B. thuringiensis,* may be unable to control all of the pest species. In that case, using methods regularly employed in chemical control programs, it will be necessary to try combinations of different microbial materials, mixtures of pathogens with compatible selective insecticides, or supplemental applications of chemical materials by themselves in order to protect the crop.

XIV. Applied Use of Entomogenous Microorganisms

There have been few reports of the utilization of insect pathogens in combination with insect parasites, predators, other microorganisms, or chemical insecticides, and studies of the direct application of insect pathogens for quick reduction of a pest population have, for the most part, been limited to the bacteria and the viruses. Experimental work with these and other types of microorganisms is expanding at a rapid pace throughout the world, and practically all groups of pathogens are under investigation for the determination of their potential as microbial control agents.

A. INTRODUCTION AND COLONIZATION

Baird (4), in discussing the use of fungus diseases in the biological control of insects, states that fungi may be utilized to control insects with three ends in view: introduction where the fungus does not occur naturally; application to initiate the disease before it would normally occur; and distribution where the saturation point for fungus and insect has not been attained under natural conditions.

A number of successful attempts at introduction and colonization of entomogenous fungi have been made. Dustan (12–14) introduced entomophthoraceous fungi into populations of the green apple bug, *Lygus communis* var. *novascotiensis* Knight, and the apple sucker, *Psylla mali* (Schmdb.), in Canada by the placement of infected adults in infested trees. A similar technique was used by Hall and Dunn (22) to spread laboratory-reared and field-collected fungi for the control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California. In each effort at control by introduction and colonization, the fungi, following successful introduction, tended to spread from the point of establishment. This spread was gradual and was dependent upon the abundance of the host insects in successive generations as well as on favorable temperature and humidity conditions in the microenvironment. Fungi that are successfully established in this manner display a remarkable ability to survive adverse conditions, and in general additional colonizations into an area are not necessary since the organisms continually reappear when conditions become favorable for their growth and a population of a susceptible host is present.

One of the classic examples of effective microbial control by the colonization of an organism in the United States has involved the mechanical dissemination of commercially produced spore powders of the milky-disease bacteria for the long-term control of the Japanese beetle. This method of distribution, utilized on turf where quick control of the pest larvae is not deemed necessary, has resulted in permanent control of the beetle in many areas within three years after introduction. The permanency of the method is one of its outstanding features, since, once the bacterial spores are introduced into an infested area, they are able to persist for long periods of time because they are periodically replenished by the infection of new generations of invading larvae.

Although the great importance of virus diseases as natural control factors has long been recognized and the possibilities of using insect viruses to control outbreaks of insect pests have been considered on many occasions, only in the last few years have the viruses been used successfully in control programs. Bergold (7) distinguishes two distinct situations in the use of insect viruses for the biological control of noxious pest species—the introduction of viruses not present in an insect population and the dissemination of viruses already present.

Much of the successful work on the introduction of viruses has been accomplished in Canada where efforts to develop economical means for controlling major pests of forest trees have had far-reaching results. Balch (5) was the first to succeed with the introduction of a virus disease when he introduced the polyhedrosis virus of the European spruce sawfly, *Diprion hercyniae* (Htg.), into insect populations in Newfoundland where it had never been observed, although the virus had given good control of outbreaks on the Canadian mainland for several years. The first successful introduction of a virus disease previously not present on the North American continent was made by Bird (8), who used ground and air application equipment to disseminate a polyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Geoff.), obtained from Sweden, into heavily infested pine plantations in Ontario, Canada. Similar studies were made in the eastern United States in 1951 and 1952 by Dowden and Girth (10). Because of these successes, according to Bergold (7), the virus has largely replaced other methods for controlling the pest in Canada and in many parts of the United States.

B. APPLICATION FOR SHORT-TERM CONTROL

Notwithstanding only limited exploration up to the present time, it is possible that the viruses may some day prove to be quite useful in the control of insect pests of truck and field crops. One of the most successful microbial control efforts against an insect on a field crop has involved the use of a polyhedrosis virus for control of the alfalfa caterpillar in the Dos Palos area of California. According to Steinhaus and Thompson (38,42), the polyhedrosis is an important factor in the natural reduction of populations of the insect. However, the natural epizootics cannot, in general, be depended upon to occur with any degree of regularity to assure satisfactory economic control of the caterpillar. Following extensive field testing involving both ground and air applications, they concluded that the virus applied as a spray is capable of initiating epizootics of disease, even in low density populations of caterpillars, and can markedly reduce the numbers of worms in a field much earlier than would occur naturally, thus reducing the amount of damage done to the crop by the insect.

They considered application by airplane or ground equipment of a virus suspension containing 5 million polyhedra per ml. at the rate of 5 gallons per acre as adequate to insure infection of a field population of the alfalfa caterpillar and its reduction below economic levels, at least under conditions usually encountered in the northern San Joaquin Valley of California. Since this study was completed in 1950, the recommended measures for control of the alfalfa caterpillar in California (Bacon *et al.*, 2) have included the application of the virus along with early cutting and chemical treatment. However, since no virus material has become available through commercial channels, the problem of supply has almost completely curtailed the use of the organism. In recent years, the virus has been applied only on a few ranches in California where personnel trained in pest control procedures have taken the time to collect or produce enough material for their own use.

There has been an upsurge of interest in the United States in the use of entomophilic nematodes for the control of insect pests under the stimulation of the finding of a new nematode parasite of the codling moth, *Carpocapsa pomonella* (L.), as reported by Dutky *et al.* (16–18). Although first observed in diseased codling moth larvae, the nematode is known to attack at least 35 other insect species. According to Dutky (16), the disease is primarily a septicemia caused by a bacterium carried into the host by the nematode. The bacterium does not infect insects readily when ingested, but when introduced by the nematode into the body cavity of the host it flourishes and not only kills the insect but also serves as food for the nematode. The bacterium produces an antibiotic that restricts other bacterial microflora in the killed insect, thus protecting the cadaver from decay that would interfere with the development of the nematode.

The ensheathed second stage nematodes can survive for long periods in the absence of suitable hosts and food. These infective stage larvae seek out the host insect, enter usually through the mouth of the host, penetrate the intestinal wall, discard their sheaths, and inject bacteria. A septicemia occurs within 24 hours. The larvae then feed on the bacteria and reproduce, and the young nematodes emerge from the dead host ready to attack a new host. The infective stages can migrate for considerable distances over a moist surface in search of a host insect and are capable of forming their own film of water over dry areas when the humidity is high. They also can migrate within a matter of hours into the center of tightly headed vegetables and can remain alive for long periods until a host is contacted. The need for moisture may limit the ability of the nematode to control insects that feed on the exposed portions of plants. However, this is partially offset by the habit of staying in the host cadaver until free water becomes available. Dutky (16) has observed that nematodes can remain alive in partially dried cadavers in the laboratory for up to 2 months. The nematodes were found to be most active at moderate temperatures (15–27°C.). In the temperature range of 24–30°C. the life cycle is 8 days, and at lower temperatures the cycle lengthens.

The results of tests in the field by Dutky and his co-workers indicate that 60–70% kill of the codling moth and the corn earworm, *Heliothis zea* (Boddie), can be achieved with applications of the nematode. This would not be considered to be satisfactory control of these insect pests in many areas, but it is likely that there are some situations where the nematode and its associated bacterium can be used successfully.

The greatest interest in applied insect pathology at this time is focused on the investigations of the commercial production and utilization of the sporeforming, crystal-bearing bacterium *Bacillus thuringiensis* for the control of insect pests. The bacterium has been known for many years since it was first isolated from diseased larvae of the Mediterranean flour moth, *Anagasta kühniella* (Zeller), in Europe in 1911. However, it was not until 1956, following the appearance of timely articles on "living insecticides" and the "potentialities of microbial control" by Steinhaus (33,35), that industrial concerns began to show an interest in the use of insect pathogens and commercial investigations into the possibilities of *B. thuringiensis* were started. In the past four years several firms in the United States have undertaken research and development studies of the bacillus, and materials containing the organism have become available for wide-scale testing against a great number of susceptible insect pests throughout this country and abroad.

B. thuringiensis is ideally suited for its role as the first organism to be widely exploited as a microbial insecticide. The bacillus is one of a group of entomogenous bacteria that form crystal-like inclusions on artificial media during sporulation. With the pathogenicity against most susceptible insects being invested in the proteinaceous inclusions rather than in the germinating spores, the use of *B. thuringiensis* as a stomach poison insecticide in control programs will be in essence the application of a toxic material contained in the inclusions, which are the product of a living organism. Thus, it will be recognized by some entomologists as a form of chemical control, although the use of the "crystals" is considered by most insect pathologists as merely another aspect of biological control.

The outstanding advantages of *B. thuringiensis* are: (*1*) it is harmless to man, other warm-blooded animals, fish, and plants, and therefore creates no residue problems; (*2*) it is specific for certain insect pests and has no effect on insect parasites, predators, and bees; (*3*) it appears to be compatible with most chemical insecticides; and (*4*) the material is easy to produce by fermentation processes and offers no storage problems since the dry powder is indefinitely stable at moderate temperatures.

The various products that have become available for field testing are composed of viable spores and inclusions of *B. thuringiensis* blended with an inert carrier. In this early period of development, the method of analysis used by the different companies is by a count of viable spores, with the quantity of inert ingredient being varied to attain a uniform spore count in the final product. However, because of the recognized importance of the inclusions, efforts are being made to develop suitable methods for the determination of the concentration of these bodies for the proper establishment of precise levels of insecticidal activity. When techniques are finally established, these counts will be implemented by bioassays against standard test insects, such as the salt-marsh caterpillar, *Estigmene acrea* (Drury), or maggots of the house fly, *Musca domestica* L., and the spore-inclusion preparations then will be accurately adjusted to meet standard activity values.

The initial materials distributed in the United States in 1958 for very limited field studies in general were wettable powder products for spray application. However, early tests by Hall and Andres (21) have indicated the desirability of dust formulations in certain instances when thorough coverage of difficult to spray leaf surfaces was necessary to obtain satisfactory kill. It appears now that most of the various *B. thuringiensis* products that are being developed will include both wettable powder and dust formulations, thereby broadening the usefulness of the microbial material.

The various products that have appeared to date have varied greatly in concentration when this is estimated only by the count of viable spores per unit of dry weight; no specific levels of toxicity of the materials have been established. Since there has been no agreement for product standardization, each manufacturer has selected a spore count level for his final product that would fit in with his method of production. In some instances, this has resulted in the development of materials of relatively low spore count (and possibly low toxicity). These preparations have appeared to be satisfactory for processing into certain dust materials, but they were too dilute and contained too large a percentage of inert carrier to be suitable as wettable powder formulations except against highly susceptible insects. It is evident that industry-wide standardization must be forthcoming if a microbial control material such as *B. thuringiensis* from more than one commercial source is to be accepted for wide-scale use in insect control programs.

The list of insects susceptible to the bacillus is quite large at this time (Hall and Dunn, 23; Steinhaus, 36; Tanada, 40) and should increase as the organism is subjected to additional laboratory and field testing. Some of these susceptible insects are major pests of crops in various parts of the world and are becoming increasingly difficult to kill with chemical materials because of developing resistance. It is quite possible that, as research continues, *B. thuringiensis* may be found to be of use in some of these programs. To be successful, however, this microbial material with its stomach poison characteristic and resultant thorough coverage requirement will have to fit efficiently into present-day control methods with a minimum of adjustment and will have to compete in terms of cost with many of the acceptable chemical insecticides.

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SYNTHETIC PYRETHROIDS

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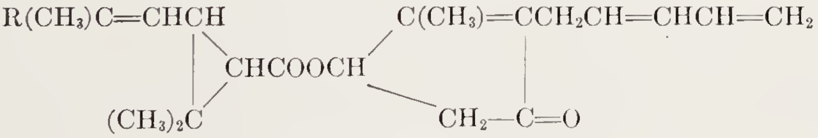
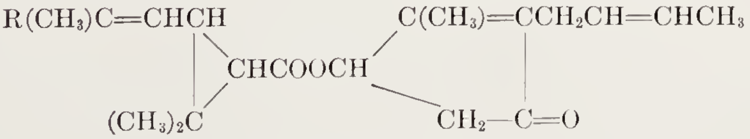
I. Introduction

Synthetic pyrethroids are compounds related to the natural insecticidal constituents of the extract of pyrethrum flowers, pyrethrins I and II (I, R = CH_3 and $COOCH_3$, respectively) and cinerins I and II (II, R = CH_3 and $COOCH_3$, respectively). Discussion of the nat-


(I)

Pyrethrin I: R = CH_3
Pyrethrin II: R = $COOCH_3$

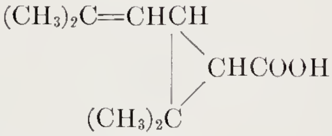

(II)

Cinerin I: R = CH_3
Cinerin II: R = $COOCH_3$

ural insecticides in this paper will be limited to comparisons between synthetic and natural pyrethroids. No attempt will be made to go into the proof of structure or other synthetic work on the pyrethrins themselves as this represents a study in itself, and is too voluminous a subject to be covered here. Neither will synergists be discussed here, except where comparisons are made between different synthetic pyrethroids to show the difference in response of the synthetic materials to synergism. Those compounds discussed in this paper are derived in part from either the acidic or the keto alcoholic component of the pyrethrins and from which they take the title "pyrethroids."

In connection with their work during the 1920's on the structure of pyrethrins, Staudinger and Ruzicka (136) prepared about 100 synthetic compounds in which they replaced the various acidic and alcoholic components of the pyrethrins with structurally related constituents. Only a few of these showed any promise, and since there was no satisfactory method of commercial synthesis, the work was practically forgotten.

Harvill (66), in 1939, prepared 22 esters of natural chrysanthemumic acid (III), some of which showed considerable activity against *Aphis rumicus*, but little further was done with them because the natural acid component was too difficult to obtain under wartime conditions and no commercial material was available.



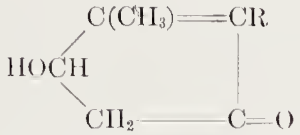
(III)

Chrysanthemumic acid

As late as 1943, Owen (118), in a report on pyrethrin insecticides, dismissed the work on synthetic analogues with only a short paragraph.

II. Cinerin I and Its Isomers

Interest was revived in study of synthetic pyrethroids when LaForge and Barthel (92) isolated cinerolone (IV, R = $CH_2CH=CHCH_3$) and elucidated the structure of the side chain of this new and less complex keto alcohol component of pyrethrum.



(IV)

Cinerolone: R = $CH_2CH{=}CHCH_3$

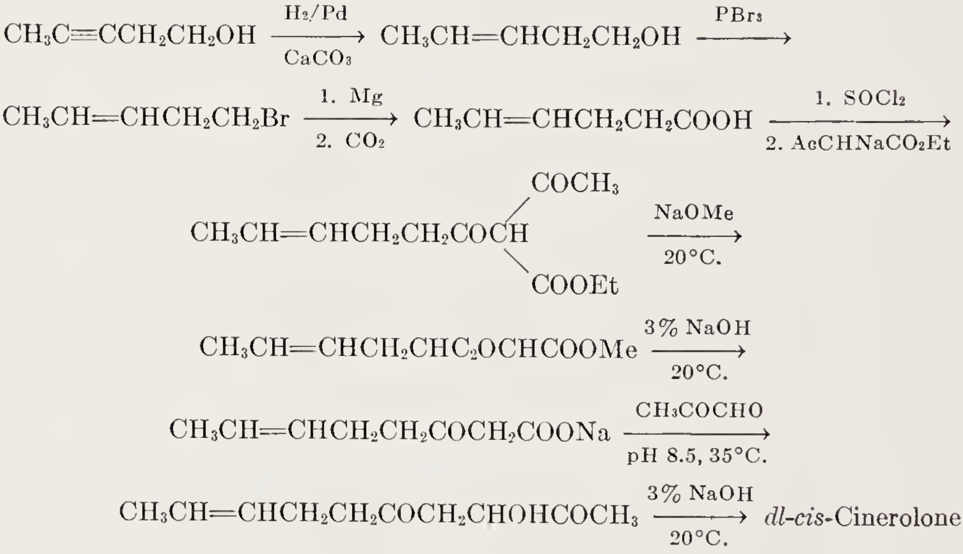
LaForge and Soloway (98) later showed that the hydroxyl of cinerolone was allylic to the ring double bond. LaForge and Barthel (93) synthesized cinerins I and II and partially hydrogenated derivatives of the cinerins and pyrethrins from fragments of natural material. Gersdorff (36) found that the toxicity to house flies of the synthetic compounds was of the same order as natural pyrethrins. He gave the following approximate relationships:

1. Pyrethrin I was 4.3 times as toxic to house flies by this method as Pyrethrin II.
2. Cinerin I was 4.0 times as toxic as cinerin II.
3. Pyrethrin I was 1.4 times as toxic as cinerin I.
4. Pyrethrin II was 1.3 times as toxic as cinerin II.
5. Pyrethrin I was 2.0 times and cinerin I was 1.4 times as toxic as the mixture of "pyrethrins" (55% pyrethrin I and cinerin I) contained in the average pyrethrum-kerosene extract.
6. Pyrethrin II was 0.46 and cinerin II was 0.35 times as toxic as this mixture.
7. Isodihydropyrethrin I was 1.8 times as toxic as isodihydrocinerin I.
8. Isodihydropyrethrin I was about as toxic and isodihydrocinerin I was about 0.6 times as toxic as the pyrethrum standard.
9. Pyrethrin I and cinerin I were about twice as toxic as their isodihydro derivatives.
10. The toxicity of isodihydropyrethrin II and isodihydrocinerin II was negligible at the concentration levels of the pyrethrum standard tests.
11. Tetrahydropyrethrin I had low toxicity as compared with the pyrethrum standard—less than 6.25% as much.

It was apparent from these observations that further study of pyrethroids might yield valuable knowledge and possibly insecticides of commercial significance.

Soloway and LaForge (131) succeeded in synthesizing 1-butyl-5-hydroxy-3-methyl-2-cyclopenten-1-one (IV, R = C_4H_9), but their method did not work when they attempted to prepare the synthetic pyrethroid analogous to cinerolone, having the unsaturated side chain. Later, in a brilliant work, Schechter, Green, and LaForge (125, 126) succeeded in synthesizing cinerolone. Schechter and LaForge were granted patent rights for this synthesis which was the first general synthesis of cyclopentenolones. Gersdorff (37) esterified the synthetic cinerolone with chrysanthemumic acid and tested the resulting synthetic compounds on house flies. He concluded that synthetic cinerolone, when esterified with the natural acid (*d-trans*-chrysanthemumic acid, III), was as toxic as natural cinerin I and 1.5 times as toxic as the natural pyrethrum mixture.

Crombie and Harper (21–23) undertook the total synthesis of cinerin I, identical with the natural material. The earlier work of Schechter *et al.* (125) had produced only a mixture of geometric and optical isomers of *trans*-cinerin I. Crombie and co-workers followed unequivocal methods in order that they would be sure of the geometric relationship in the side chain of the cyclopentenolone ring. They proceeded as shown in Scheme 1.



$$CH_3C{\equiv}CCH_2CH_2OH \xrightarrow[CaCO_3]{H_2/Pd} CH_3CH{=}CHCH_2CH_2OH \xrightarrow{PBr_3}$$

$$CH_3CH{=}CHCH_2CH_2Br \xrightarrow[2.\ CO_2]{1.\ Mg} CH_3CH{=}CHCH_2CH_2COOH \xrightarrow[2.\ AcCHNaCO_2Et]{1.\ SOCl_2}$$

$$CH_3CH{=}CHCH_2CH_2CO\underset{COOEt}{\overset{COCH_3}{CH}} \xrightarrow[20°C.]{NaOMe}$$

$$CH_3CH{=}CHCH_2CHC_2OCHCOOMe \xrightarrow[20°C.]{3\%\ NaOH}$$

$$CH_3CH{=}CHCH_2CH_2COCH_2COONa \xrightarrow[pH\ 8.5,\ 35°C.]{CH_3COCHO}$$

$$CH_3CH{=}CHCH_2CH_2COCH_2CHOHCOCH_3 \xrightarrow[20°C.]{3\%\ NaOH} \textit{dl-cis}\text{-Cinerolone}$$

Scheme 1

By esterifying *dl-cis*-cinerolone with *d-trans*-chrysanthemumic acid, Crombie *et al.* (22) obtained a material almost identical with the natural, thereby proving that the side chain of the natural product was of *cis* configuration. They also confirmed the high order of toxicity to insects of the synthetic cinerins.

Schechter, Green, and LaForge (126) also prepared *dl-cis*-cinerolone and showed that natural cinerolone was of *cis* configuration.

Cupples (26) compared the infrared spectra of cinerin I and its *trans* isomer. He showed that infrared offers a good means of differentiating between the *cis* and *trans* isomers of the cyclopentenolones. He was able to confirm Schechter, Green, and LaForge's (125) assumption that their synthetic 2-(2-butenyl)-4-hydroxy-2-methyl-2-cyclopenten-1-one was a *trans* isomer of the natural (*cis*) material.

LaForge and Green (95) synthesized all four optical isomers of cinerin I. The results of tests of these isomers against house flies are given in Table I.

TABLE I
Toxicities of Cinerins to House Flies (95)

Ester components		
cis-Cinerolone	*trans*-Chrysanthemumic acid	Relative toxicity[a] (pyrethrin = 1.0)
d	*d*	0.67
l	*d*	1.2
d	*l*	.17
l	*l*	.12
Pyrethrin standards		1.0

[a] Campbell turntable method.

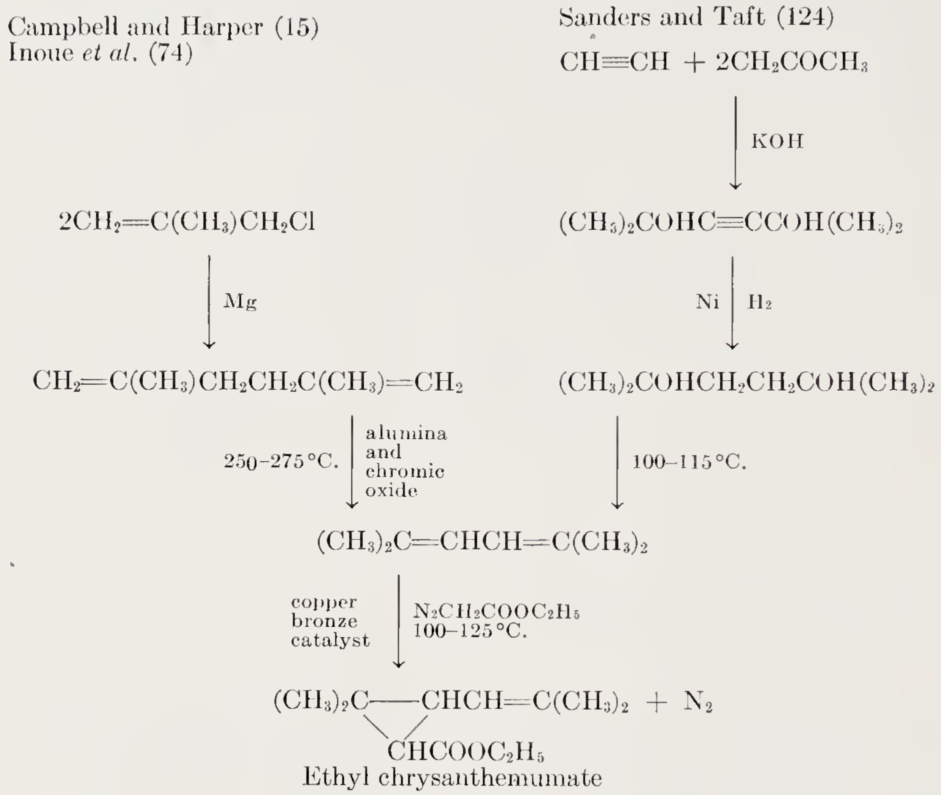
Campbell and Mitchell (17) showed that polymerization of pyrethrins and cinerins is confined to the keto alcoholic portions of the molecule and that the chrysanthemum acids themselves are unchanged. This is important in that it suggests the synthetic pyrethroids are inherently more stable than the natural pyrethroids. The synthetics usually consist of a more stable alcoholic component esterified with the stable chrysanthemumic acid. Most synthetic pyrethroids so far studied are derivatives of chrysanthemumic acid. When the keto alcohol configuration exists in the synthetic, it is of a stable variety for the simple reason that unstable ones are too hard to synthesize except for structural studies.

Harper (61), in an excellent review, brought up to date the synthetic studies made on the pyrethrins.

III. Chrysanthemumic Acid

One of the most significant steps in the study of synthetic pyrethroids was the synthesis of chrysanthemumic acid by Campbell and Harper (15), who adapted the synthesis of Staudinger and Ruzicka

(134) to essentially that now used commercially (124), except for the preparation of the diene hydrocarbon starting material (Scheme 2).


Campbell and Harper (15)
Inoue *et al.* (74)
Sanders and Taft (124)
CH≡CH + 2CH₂COCH₃
KOH
(CH₃)₂COHC≡CCOH(CH₃)₂
Ni
H₂
(CH₃)₂COHCH₂CH₂COH(CH₃)₂
100–115 °C.
2CH₂=C(CH₃)CH₂Cl
Mg
CH₂=C(CH₃)CH₂CH₂C(CH₃)=CH₂
250–275 °C.
alumina and chromic oxide
(CH₃)₂C=CHCH=C(CH₃)₂
copper bronze catalyst
N₂CH₂COOC₂H₅
100–125 °C.
(CH₃)₂C——CHCH=C(CH₃)₂ + N₂
CHCOOC₂H₅
Ethyl chrysanthemumate

Scheme 2

By saponification of the ethyl chrysanthemumate Campbell and Harper (15) obtained a mixture of the higher melting (116°C.), less soluble *cis*- and the lower melting (54°C.), optically inactive *trans*-chrysanthemumic acids. After crystallization and removal of the *cis* acid, the *d,l-trans* acid was separated from the residue by distillation and resolved into its components, the *d-trans* and *l-trans* acids, via the quinine salt. Comparisons of the melting points and specific rotations of the amides, anilides, and *p*-phenylphenacyl esters established the identity of the *d-trans* acid with the acid obtained from natural material.

Harper, Reed, and Thompson (62,64) later reviewed the previous work and reported on improvements in methods and materials, particularly in the use of tetrahydrofuran as a solvent for the reaction between magnesium and methallyl chloride.

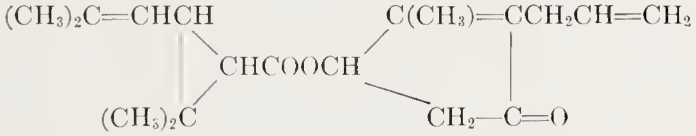
Inoue and staff (73,74,87,88), in Japan, made an excellent study of the synthesis of what they called "synthetic pyrethrins," but which are more properly called synthetic pyrethroids. They examined first the synthesis of chrysanthemumic acid by methods best suited to Japan. Instead of the acetylene–acetone synthesis used in the United States for the preparation of 2,5-dimethyl-2,4-hexadiene, they examined ethyl levulinate, diethyl succinate, and methallyl chloride as starting materials. They finally settled on the methallyl chloride route used by Campbell and Harper.

The derivatives are the subject of a British patent (115).

IV. Allethrin and Its Analogues

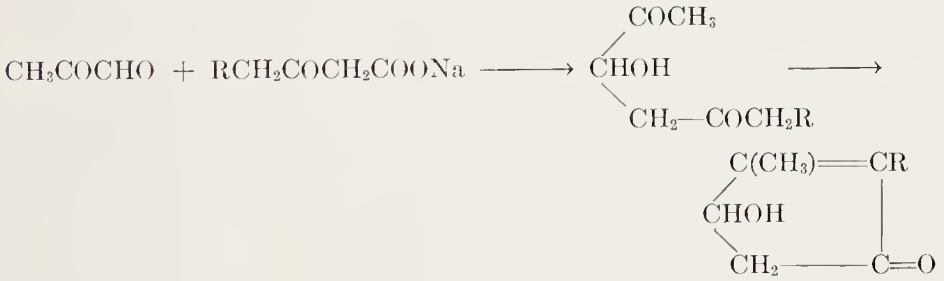
A. ALLETHRIN

Allethrin (V) is the most prominent of the class of synthetics derived from the cyclopentenolone ester of chrysanthemumic acid. The members of this series differ only in the substituents on the cyclopentenolone and/or cyclopropane rings.



(V)
Allethrin

Allethrolone was first synthesized by Schechter, Green, and LaForge (125,128) by the unique method which was to set the pattern for the general synthesis of cyclopentenolones:



Allethrolone: R = allyl

R may be aliphatic (straight chain or cyclic) or aryl

According to Gersdorff (37), when the cyclopentenolone with the allyl side chain was esterified with the natural chrysanthemumic acid, the product was 6 or 7 times as toxic to house flies as the natural pyrethrins mixture. The wholly synthetic allyl analogue (V), now called allethrin was 1.8 times as toxic to house flies as the standard pyrethrins and was considered the most toxic to house flies of the synthetic pyrethroids.

In another study LaForge and co-workers (94) observed that allethrin made with *l-trans*-chrysanthemumic acid was only 2% as toxic to house flies as that made with the *d-trans* acid. Isomerism, optical and geometric, was responsible for considerable variations in biological activity of allethrin. LaForge, Green, and Schechter succeeded in synthesizing and resolving the eight possible isomers after a long and difficult study; LaForge worked several years after his retirement, without compensation from the United States Government, to finish this task. Following in general a procedure like that used on cinerolone (95), these workers prepared the esters of *dl*-allethrolone with the natural *d-trans*-chrysanthemumic acid and separated the semicarbazones of the esters by their differential solubility in ether–petroleum ether mixtures. Methanolysis of the separated ester semicarbazones gave the *d* and *l* isomeric forms of allethrolone semicarbazone. The semicarbazones were hydrolyzed to the keto alcohols by use of potassium acid sulfate in a two phase water–petroleum ether solution. Acylation with *d* and *l* isomeric forms of *trans*-chrysanthemumic acids gave the four optically active *trans* acid isomers of allethrin (Table II).

TABLE II
Optically Active Esters of Allethrolone with *trans*-Chrysanthemumic Acid (96)

Allethrin components				Analysis[b]	
Allethrolone	*trans*-Chrysanthemumic acid	n_D^{25}	$[\alpha]_D^{25}$[a]	C, %	H, %
d	*d*	1.5050	−19.4	74.78	8.41
d	*d*[c]	1.5047	−22.3	—	—
l	*d*	1.5047	+22.4	75.56	8.51
d	*l*	1.5046	−22.6	75.75	8.62
l	*l*	1.5040	+17.0	75.60	8.62
l	*l*[c]	1.5034	+20.9	—	—

[a] In purified kerosene (Deobase).

[b] Calculated for $C_{19}H_{26}O_3$: C = 75.46%; H = 8.67%.

[c] Prepared by sulfuric–acetic acid method. All others were prepared by resynthesis.

Using the method of Campbell and Harper (16) for the resolution of *dl-cis*-chrysanthemumic acid, LaForge and co-workers (97) completed the series by preparing the *d*- and *l*-allethrolone esters of *cis*-chrysanthemumic acid. The properties are given in Table III.

TABLE III
Optically Active Esters of Allethrolone with *cis*-Chrysanthemummonocarboxylic Acid (97)

Allethrin components				$[\alpha]_D^{25}$		Analysis[b]	
Allethrolone	*cis* acid	Boiling point, °C.	n_D^{25}	Kerosene[a]	No solvent	C, %	H, %
d	*d*	142–148/0.25 mm.	1.5080	±5.6	±13.5	74.79	8.47
l	*l*	135–140/0.05 mm.	1.5070	−5.5	−14.8	75.08	8.59
d	*l*	144–146/0.1 mm.	1.5074	−19.7	−19.4	74.59	8.84
l	*d*	147–148/0.1 mm.	1.5076	±19.1	±19.0	75.19	8.57

[a] In purified kerosene (Deobase), *c* 16.

[b] Calculated for $C_{19}H_{26}O_3$: C = 75.46%; H = 8.67%.

Gersdorff and co-workers (39,43,49,50) then evaluated these isomeric materials by comparison with each other and with standard allethrin (purified technical allethrin from commercial source). They also studied the effect of piperonyl butoxide, a synergist, on the *cis*

TABLE IV
Toxicities of Isomers of Allethrin (50)

Optically active isomer	Percentage in allethrin	Relative toxicity (allethrin = 1.0)	
		Expected	Found
ld-trans	12.4		0.579[a]
dl-trans	12.4		0.138[a]
dd-trans	22.8		3.367[a]
ll-trans	22.8		0.0224[a]
ld-cis	8.0		0.327
dl-cis	8.0		0.138
dd-cis	6.8		1.775
ll-cis	6.8		0.0565
Composite	100	1.023	1.0

[a] Gersdorff and Mitlin (43).

TABLE V

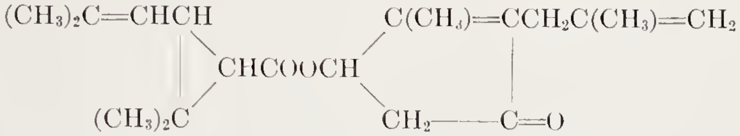
Relative Toxicities of *cis* and *trans* Fractions of Allethrin and Effect of Piperonyl Butoxide as Synergist (49)

				With piperonyl butoxide		
	Allethrin	*trans*-Allethrin	*cis*-Allethrin	Allethrin	*trans*-Allethrin	*cis*-Allethrin
LC_{50}, mg./100 ml.						
Original basis[a]	57.63	53.46	105.6	20.47 + 204.7	17.86 + 178.6	36.29 + 362.9
Pyrethroid equivalent basis	57.63	53.46	105.6	22.64	19.62	43.34
Allethrin equivalent basis	57.63	57.63	57.63	22.64	21.15	23.65
Relative standard error of original LC_{50}, %	4.52	4.59	4.50	4.15	4.11	4.10
Relative toxicity (allethrin = 1)						
Principal toxicant alone	1.0	1.078 ± 0.069	0.546 ± 0.035	1.0	1.078	0.546
Mixture on pyrethroid equivalent basis				2.545 ± 0.190	2.937 ± 0.218	1.330 ± 0.096
Intensity of synergism at 50% mortality level				2.55 ± 0.19	2.72 ± 0.20	2.44 ± 0.18
Log of intensity				0.406	0.435	0.387
Minimum log required to demonstrate synergism				0.0634	0.0633	0.0612
Range of intensity ratio between 5 and 95% levels				1.8–3.6	1.9–3.9	1.7–3.5

[a] For the compound expressions the first figure refers to the pyrethroid.

and *trans* fractions of allethrin. Table IV gives the relative toxicity of these stereoisomers, and Table V the comparison of *cis* and *trans* fractions and the intensity of their synergism with piperonyl butoxide.

Fales *et al.* (33) compared allethrin, its methallyl analogue (VI), purified natural pyrethrins (11), and pyrethrum extract in conjunction with DDT in aerosols and also in sprays against house flies and roaches.



(VI)

Methallyl analogue of allethrin

Results of these tests are given in Tables VI, VII, and VIII. The stability of the synthetic materials is emphasized in this report. The same workers (29) made storage tests on allethrin aerosol formula and found no deterioration in effectiveness after 15 months.

TABLE VI

Comparison of Allyl Ester, Allethrin, and Pyrethrins in Aerosols and Sprays against House Flies (33)

(In per cent)

Material	Knockdown in 5 min.	Knockdown in 10 min.	Knockdown in 15 min.	Kill in 1 day
Aerosols (6 tests)				
Allyl ester[a] (1%)	84	93	99	75
Allethrin (1%)	61	83	92	53
Pyrethrins (1%)	64	79	86	44
Sprays (5 tests)				
Allyl ester[a] (1 mg./ml.)	82	91	—	69
Allethrin (1 mg./ml.)	65	82	—	39
Pyrethrins (1 mg./ml.)	75	83	—	40

[a] Natural acid ester.

Allethrin has been compared with pyrethrins against house flies in a great many studies (22,29,32,35–37,43,45–47,51–53,55,69,86, 109,111,112,120). Perhaps one of the most distinguishing features of allethrin-pyrethrin comparisons has been disagreement among the

TABLE VII

Effect of Substituting Synthetic Esters for Natural Pyrethrins in an Aerosol[a] in Tests against House Flies (33)

(Average of three tests at 1.16 g. of aerosol/1000 cu. ft., in per cent)

Ester or pyrethrins	Knockdown in 5 min.	Knockdown in 10 min.	Knockdown in 15 min.	Kill in 1 day
Allyl ester	61	72	85	93
Methyllallyl ester	55	73	85	94
Pure natural pyrethrins	16	37	52	88
Pyrethrins from pyrethrum extract	20	44	61	93

[a] Aerosol formulas: synthetic ester or pyrethrins, 0.4%; DDT, 3%; cyclohexanone, 5%; lubrication oil, 5%; deodorized kerosene, 1.6%; Freon-12, 85%.

various workers with the same, and with different, methods of testing. Moore (109) reported on Peet-Grady tests on house flies in several laboratories. The divergent results listed in Table IX clearly show the effects of differences in technique, materials, methods, and population of flies. After a number of comparisons in his own and other laboratories, with allethrin from various sources, Moore summarized his work as follows:

> The allethrin has been compared with natural pyrethrins against a variety of insects in several different formulations. The allethrin was found to be about as toxic as pyrethrins to house flies (*Musca domestica*) and about one-third to one-half as toxic as pyrethrins to cockroaches and a number of field insects.
>
> It has been pointed out by other workers that higher concentrations of the allethrin, as compared with natural pyrethrins, indicate that the allethrin is more toxic than the natural pyrethrins. In none of the above tests were concentrations in this neighborhood used. Therefore, it was not shown that the allethrin is more toxic than the pyrethrins at these levels.
>
> It has been shown that the allethrin is fully as toxic as the pyrethrins when used against such insects as house flies and less toxic when used against such insects as cockroaches and some field insects. This would indicate that the allethrin could replace the natural pyrethrins in aerosol bombs and as fly spray.
>
> The use of some of the synergists indicates that the allethrin might well be used when in combination with these synergists to replace the natural pyrethrins on control work of cockroaches and certain field insects. Further work along these lines is necessary before definite conclusions can be drawn.
>
> In conclusion, it may be stated that the allethrin has a definite position in the insecticide industry and as more is learned about this product, it will become one of the more important discoveries in the industry.

TABLE VIII
Effect of Synthetic Esters Substituted for or Allyl Ester Added to U. S. Public Health Service Formula in Tests against American Cockroaches (33)
(Average of two tests at 23.15 g. of aerosol/1000 cu. ft., in per cent)

Aerosol formula	Concentration	Kill in 5 days	
		Adult females	Large nymphs
Public Health Service Formula			
Pyrethrum extract (20%)	5	85	20
DDT	3		
Cyclohexanone	5		
Lubricating oil	2		
Freon-12	85		
Allyl ester substituted			
Allyl ester	1	45	25
DDT	3		
Cyclohexanone	5		
Deobase	4		
Lubricating oil	2		
Freon-12	85		
Methylallyl ester substituted			
Methylallyl ester	1	5	16
DDT	3		
Cyclohexanone	5		
Deobase	4		
Lubricating oil	2		
Freon-12	85		
Allyl ester added			
Pyrethrum extract (20%)	5	100	30
DDT	3		
Allyl ester	1		
Cyclohexanone	5		
Lubricating oil	1		
Freon-12	85		

Stoddard and Dove (137) were among the first to demonstrate the effect of differences in methods of assay and concentration on comparison of allethrin with pyrethrins. Their results by the Campbell turntable method would indicate that allethrin and pyrethrins are about equally toxic to house flies at very low dosages (50 mg./100 ml.) and kill (5%). Above this level, allethrin is more toxic than pyrethrins. When these two materials were compared by the Peet-Grady method, the equivalence dosage was approximately

TABLE IX

Comparative Toxicity of Allethrin and Pyrethrins in Peet-Grady Tests on House Flies (109)

Manufacturer	Lab.	Pyrethrins, g.	Allethrin, g.	Kill in 24 hrs., %
—	D	0.0120	—	48
3	D	—	0.0120	11
—	E	0.0120	—	34
2	E	—	0.0120	43
4	A	—	0.0120	23
—	A	0.0120	—	14
4	A	0.0080	0.0040	12
3	A	—	0.0120	6

125 mg./100 ml. and equivalence kill was approximately 50%. At lower dosages and kill, pyrethrins were more toxic than allethrin. Improvements in technique and methods seem to be required because, as things stand, the simple statement as to which insecticide is superior against a certain species is impossible without identification of methods, concentration, and dosage.

Elliott, Needham, and Potter (28), in an interesting study comparing allethrin, the pyrethrins, and allethrolone esterified with natural *d-trans*-chrysanthemumic acid, found that the relative toxicity of these insecticides varied according to the species used as test subjects. In this study they used *Plutella maculipennis* (Curt.) (diamond back moth), *Macrosiphum solanifolii* (Ashm.) (potato aphid) *Phaedon cochleariae* (Fab.) (mustard beetle), and *Oryzaephilus surinamensis* (L.) (saw-toothed grain beetle). They conclude:

> The results leave little doubt that the relative toxicity of these closely related compounds varies considerably with the different insect test subjects, the extremes for the comparison of the partially synthetic material with the natural pyrethrins being from about one-eighth as toxic with *Macrosiphum solanifolii* as test-subjects to nearly four times as toxic with *Plutella maculipennis*. For the fully synthetic material it ranges from approximately one-sixteenth as toxic as the natural pyrethrins with *Macrosiphum solanifolii* as test-subjects to nearly twice as toxic with *Plutella maculipennis*. The partially synthetic ester was almost twice as toxic as the fully synthetic one to *Plutella maculipennis* larvae, to *Macrosiphum solanifolii* and to *Phaedon cochleariae*. However, the completely synthetic material was considerably more than half as toxic as the partially synthetic one to *Oryzaephilus surinamensis*.

Mitlin and Babers (104) found that, applied topically, allethrin was as toxic as pyrethrins to flies but only 3% as toxic to the American cockroach (*Peiplaneta americana*). Blum (12,13), on the other hand, found allethrin to be only one-half as toxic to house flies as pyrethrin when applied topically. Rather than devaluating the results of the various workers, this divergence again points out the part biological variation plays in the studies.

Busvine and Nash (14) determined the potency and persistence against several insects of a number of commercial synthetic insecticides, including allethrin. Allethrin was rated with DDT and chlordane and below pyrethrins in potency. As was expected, allethrin and pyrethrins were among the least persistent of the group.

Atkins and Anderson (5) found allethrin to cause low mortality in laboratory tests with honey bees.

Fales, Bodenstein, and Piquett (30) found that the toxicity of allethrin, when compared with pyrethrins, varied between three species of mosquitoes. They report:

> Against male *Aedes*, the amount of allethrin required was again eight times that of pyrethrins. With *Anopheles* the materials gave nearly equal performance. With *Cules* the lowest concentration of allethrin (3.2 mg./ml.) gave approximately the same result as the highest concentration of pyrethrins (1.6 mg./ml.).

Nagasawa *et al.* (113) found that in an incense allethrin was more effective than pyrethrins against *Culex*, but this difference could be due to the greater stability of allethrin against the heat of incense burning. Nagasawa and his co-workers (112) also studied the action of pyrethrins and allethrin against mosquito pupae.

Allethrin has rapidly found a place in the insecticidal industry as a partial substitute for pyrethrum. It has been the subject of extensive reviews (27,58,125), particularly those of Roark (122) and, later, Roark and Nelson (123) who reported on both the chemical and insecticidal studies with allethrin through 1955.

The successful establishment of allethrin as a commercial insecticide created the need for suitable methods of analysis. The hydrogenolysis method of LaForge and Acree (91) and the ethylenediamine method proposed by Hogsett, Kacy, and Johnson (70) are the two most general methods. In addition, Feinstein (34), Cueto and Dale (25), Oiwa *et al.* (117), Green and Schechter (57), and Moore (110) have proposed methods, all of which seem to have considerable utility. These methods are reviewed by Konecky (90) and Haring (59).

When commercial allethrin is kept at 4°C. crystallization sets in, and a product of uniform composition can be isolated which consists of *l-trans*-chrysanthemumic acid esterified with *d*-allethrolone (129). When recrystallized from isooctane, the material melts at 50.5–51°C. This isomer may be useful as a reference standard because it can be obtained in a high state of purity (127). Gersdorff *et al.* (54) made extensive tests with this material to study its possibilities as a standard in bioassay work, but concluded that it offered no biometrical advantage over purified allethrin or pyrethrins, although its stability over a period of 38 months was well demonstrated.

Sanders and Taft (124) gave an excellent report on the manufacture of allethrin, describing in some detail the 18 steps necessary for synthesis. Very few products made by American chemical industry require such lengthy processing.

B. RADIOACTIVE ALLETHRIN

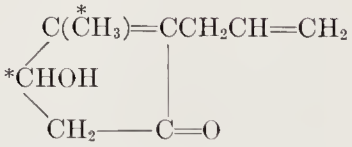
Acree, Roan, and Babers (1) prepared allethrin labeled with carbon-14 in the chrysanthemumic acid moiety and purified it by chromatographic technique to 98% pure allethrin containing *dl-cis,trans*-allethrin-*2-C*^{14}. Up to the present time their biological work with this radioactive insecticide has not been reported.

Winteringham, Harrison, and Bridges (143) prepared allethrin, labeled in the allethrolone fragment, by the following series of reactions:

$$\overset{*}{C}H_3CO\overset{*}{C}H_3 \xrightarrow{SeO_2} \overset{*}{C}H_3CO\overset{*}{C}HO$$

$$CH_2{=}CHCH_2CH_2COCH_2COOH + \overset{*}{C}H_3CO\overset{*}{C}HO \longrightarrow$$

$$CH_2{=}CHCH_2CH_2COCH_2\overset{*}{C}HOHCO\overset{*}{C}H_3 \longrightarrow$$



Pellegrini, Miller, and Sharpless (119) labeled pyrethrins biosynthetically with C^{14}.

The metabolism of these radioactive compounds was studied by treating house flies topically and resolving the metabolic products chromatographically at the end of a 24 hour incubation period. It was found that the radioactive preparations were metabolized to noninsecticidal substances within 24 hours. When the synergist piperonyl cyclonene was applied simultaneously, the metabolism was substantially inhibited. Since allethrin was metabolized to a greater degree than were pyrethrins, Winteringham *et al.* (143) speculated that more effective allethrin synergists are possible. Also, it was found that piperonyl cyclonene retarded absorption of the insecticide by the insect, presumably by dilution.

C. TOXICOLOGY OF ALLETHRIN

Starr, Ferguson, and Solomon (132) reported on toxicity tests with technical allethrin on white rats. White rats exhibited no harmful effects when fed 0.2% in diet for 24 weeks, or when a 1% aerosol mist was inhaled 1/2 hour per day, 6 days a week, for 22 weeks. Microscopic examination of the organs showed no abnormalities correlated with allethrin application.

Carpenter and co-workers (18) made a detailed study of the toxicity of allethrin and pyrethrins using white rats, guinea pigs, rabbits, dogs, and white mice as test animals. They summarize their work as follows:

> Two separate inhalation studies on aerosols containing 1% by weight of allethrin or of pyrethrins, 9% of peanut oil and 90% of Freon-12 were carried out at concentrations in excess of 50 g. of total formulation per 1,000 cu. ft. of space.
>
> In the first study laboratory-produced allethrin and the comparative aerosols caused no detectable injurious effects on rats exposed twice daily for 30 minute periods up to a total of 85 such periods within 67 calendar days. In a second similar study a sample representing commercially produced allethrin caused no injury in rats or dogs receiving 40 exposures, each of 30 minutes' duration, within 27 calendar days.
>
> Single 30 minute exposures of rats to concentrations of aerosols of commercial allethrin and pyrethrins on the order of 350 times the level used for the repeated exposures caused no visible damage, nor did they depress weight gain during a subsequent 14 day observation period.
>
> A fog of commercial allethrin produced by a Vaponefrin nebulizer was lethal to one of 10 rats in a two-hour exposure at a concentration of 19 mg./liter, and only four of 10 succumbed in a four hour exposure to 13.8 mg./liter. These massive concentrations of allethrin are respectively 10,000 and 7,000 times the amount that would be present in the aerosols utilized in freeing aircraft from insects. The extreme viscosity of an 84% pyrethrin concentrate and its unavailability made comparisons by this method impossible.
>
> The single dose acute oral LD_{50}'s of commercial allethrin for rodents fed 20% dilutions in deodorized kerosene are as follows: mice 0.48, rats 0.92, and rabbits

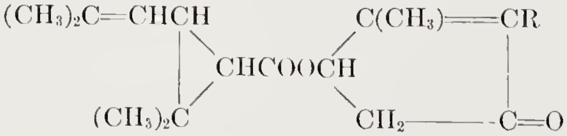
> 4.29 g./kg. Two different samples of purified pyrethrins, 20% in petroleum distillate, gave LD_{50} values of 0.82 and 1.87 g./kg. for rats.
>
> The LD_{50} of undiluted commercial allethrin for rabbits by the percutaneous route is 11.2 ml./kg. Dilution in deodorized kerosene markedly increases toxicity by skin penetration, but dimethyl phthalate appears not to aid penetration.
>
> Undiluted commercial allethrin and dilutions in deodorized kerosene are harmless to rabbit eyes, but they cause moderate erythema of the clipped skin of the rabbit belly when applied in single or repeated applications. Drill cloth impregnated with this allethrin at the rate of 4 g. per square foot caused marked erythema of the hair-free trunk of rabbits when worn for three days. Subsequently these reactions subsided even though the impregnated bands were reapplied twice each week during a 21-day period of wear. No systemic injury, as judged by weight changes, resulted and all skin reactions had subsided in this interval.
>
> Guinea pigs could not be sensitized by a course of eight intracutaneous injections of a 0.1% dispersion of allethrin in 3.3% propylene glycol in isotonic sodium chloride solution followed by a 21-day incubation period before retest.
>
> The evidence presented above indicates that commercial allethrin is of the same relative order of toxicity as pyrethrins. On the basis of this comparison we conclude that it may be used safely as an insecticide in sprays and aerosols.

Lehman (99), in his discussion of potential hazards of insecticides, tabulates for pyrethrins "Among the safest of insecticides. Toxicological hazards under conditions of use extremely remote" and for allethrin "Evidence at present indicates similar toxicity to that of pyrethrins."

Ambrose and Robbins (4) administered allethrin gastricly and subcutaneously to rats. Also, they applied it to the skin of guinea pigs and three humans. They concluded that allethrin was no more toxic than pyrethrins. Also, they noted that what little toxic reaction was observed may have been due to impurities.

D. ALIPHATIC ALLETHRIN ANALOGUES

Schechter (125,126), Gersdorff (37,40,42), Inoue (73,88), Mitsui (106,108), and Crombie (21,23), with their co-workers, examined various analogues of allethrin as shown in Table X. None of this new group equaled allethrin against house flies. Addition of chlorine to the allethrin molecule lowered the insecticidal effect.



(VII)

TABLE X
Toxicities of Allethrin Analogues (VII) to House Flies and Mosquito Larva

R in formula VII	Relative toxicity[a] (pyrethrins = 100)	Investigators
Hydrogen	Nontoxic	125
Methyl	40	42,73,88
Ethyl	94	42,73,88
Propyl[b]	—	73,88
Isopropyl	19[c]	106,108
Butyl	17[d],23[c]	37,73,88,106,125
Allyl (allethrin)	300,65[c]	21,37,88,125
Allyl	604-617[d]	21,37,88,125
Propenyl	46[c]	106,108
2-Methallyl	346[d]	37,73,88,125
2-Butenyl (synthetic cinerin I)	148[d]	23,37,125
3-Butenyl	61[d]	3,6,37,125
3-Methyl-2-butenyl	21[d]	3,6,37,125
2-Chloroallyl	156	40
3-Chloroallyl	142	40
3-Chloro-2-butenyl	21	40
2-Butenyl	73	40
2-Pentenyl	Equal to pyrethrins Peet-Grady	23

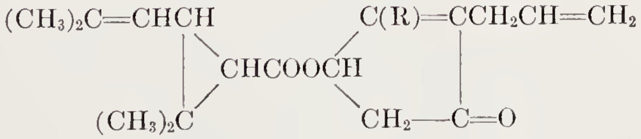
[a] Data on relative toxicity to house flies by Campbell turntable from Gersdorff (37,40,42).

[b] Data reported by Inoue (73) indicate propyl analogue to be slightly more toxic to house flies than ethyl and butyl analogues, but less toxic than the allyl analogue.

[c] Data from Mitsui and Nagase (108) on mosquito larvae (*Culex pipiens pollens*).

[d] Natural chrysanthemumic acid as acid component.

Gersdorff *et al.* (40) also reported on the analogue of allethrin with two allyl groups (VIII, R = allyl), but the diallyl analogue had little value as a fly toxicant. Neither did the phenyl derivative (VIII, R = phenyl) (37) show toxicant action.



(VIII)

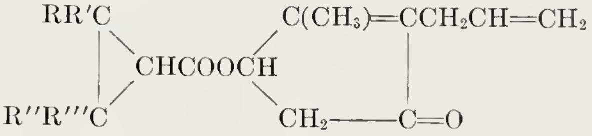
LaForge *et al.* made a study of variations in the acid component of allethrin. In Table XI is given a list of the cyclopropane acid

TABLE XI
Toxicity to House Flies of (94)

Substituents in formula IX				Relative toxicity,[a] % (pyrethrins = 100)
R	R′	R″	R‴	
CH_3	CH_3	H	$CH_2CH(CH_3)_2$	103
H	H	H	$C(CH_3){=}CH_2$	31
H	H	CH_3	$CH_2CH_2C(CH_3){=}CH_2$	6
CH_3	CH_3	H	$C(CH_3){=}CHCH_3$	13
H	H	H	$CH{=}CHCH_3$	6
H	H	CH_3	$CH_2C(CH_3)$	3
H	H	H		3
H	H	H	NO_2	16
CH_3	CH_3	H	$CH{=}C(CH_3)_2$ (*l-trans* acid)	12
CH_3	CH_3	H	$CH{=}C(CH_3)COOCH_3$ (*d-trans* acid ester)	135
CH_3	CH_3	H	$CH{=}C(CH_3)_2$ (*d-trans* acid)	594

[a] Campbell turntable method.

variations with a comparison with allethrin made with *l-trans*-chrysanthemumic acid, *d-trans*-chrysanthemumdicarboxylic acid monomethyl ester, and *d-trans*-chrysanthemumic acid. The importance of geometric configuration of the molecule is apparent.



(IX)

In another study, Gersdorff (37) had tested a compound analogous to allethrin but open chain instead of having the cyclopentene ring. This was found to have very little toxicity to house flies at four times the dosage with which allethrin gave 100% kill.

E. CYCLIC ALLETHRIN ANALOGUES

Matsui, in Japan, and LaForge, Green, and Schechter, in the United States, simultaneously prepared a new pyrethroid similar to pyrethrin I, but having a ring side chain closed through an oxygen linkage, now called furethrin (X). When the chemists involved were apprised of each other's work, they decided upon joint publication, their article appearing in 1952 (103)

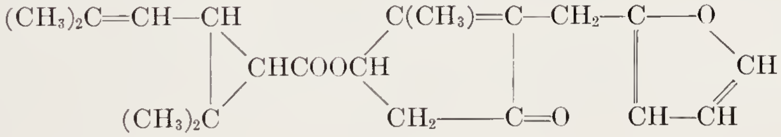
Matsui obtained Japanese patents (101,102) on related cyclopentenone derivatives and insecticides.

Gersdorff and Mitlin (41) tested furethrin against house flies. They compared the Japanese sample, which had apparently deteriorated in transit, with the LaForge sample and with pyrethrins as a standard. Also they included the natural acid isomer, with the results shown in Table XII. Fales, Bodenstein, and Piquett 31 tested furethrin and found it to be slightly less effective than allethrin against house flies, but about equal to allethrin against American cockroach and *Aedes* mosquitoes.

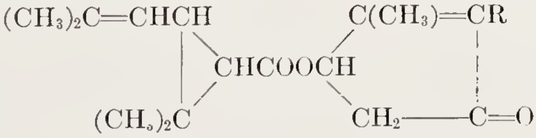
TABLE XII

Toxicities to House Flies of Furfuryl Analogue of Allethrin as Compared with Pyrethrins (41)

Material	LC_{50}, mg./ml.	Relative toxicity (pyrethrins = 1.0)
Furethrin		
Bureau (LaForge) sample	1.877 ± 0.090	1.109 ± 0.075
Japanese sample	2.872 ± 0.128	0.725 ± 0.047
d-trans acid isomer	1.087 ± 0.050	1.915 ± 0.127
Pyrethrins	2.082 ± 0.100	1.0


(X)

Chen and Barthel (19) prepared five new pyrethroids having aromatic side chains on the cyclopentenolone ring (VII, R = phenyl (phenethrin), benzyl (benzythrin), *p*-methoxybenzyl, *o*-methoxybenzyl, and 3,4-methylenedioxybenzyl). These esters were prepared with *dl-cis trans*-chrysanthemumic acid. Gersdorff and Mitlin


(VII)

Phenethrin: R = phenyl
Benzylthrin: R = benzyl

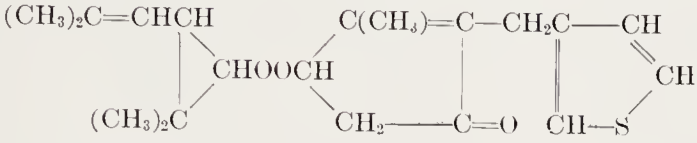
TABLE XIII

Toxicities of Some Aryl Analogue of Allethrin (44)

Material	Hetero-geneity factor	Standard error of regression coefficient	LC_{50}, mg./ml.	Relative toxicities: Allethrin = 100	Relative toxicities: Pyrethrins = 100
Series 1					
Phenyl analogue	6.47	0.38	307 ± 15	33.7 ± 1.8	95.1 ± 6.8
Allethrin	—	0.11	103.6 ± 2.6	100	282 ± 17
Pyrethrins	4.52	0.22	292 ± 16	35.5 ± 2.1	100
Series 2					
Benzyl analogue	3.75	0.17	599 ± 27	17.9 ± 1.4	56.1 ± 5.5
Allethrin	6.82	0.27	107.2 ± 6.5	100	313 ± 33
Pyrethrins	10.44	0.29	336 ± 29	31.9 ± 3.4	100
Series 3					
o-Methoxybenzyl analogue	8.95	0.30	1315 ± 117	6.93 ± 0.81	17.5 ± 1.7
3,4-Methylenedioxybenzyl analogue	24.62	0.57	1516 ± 183	6.01 ± 0.86	15.2 ± 1.9
Allethrin	7.28	0.28	91.1 ± 7.0	100	252 ± 22
Pyrethrins	—	0.12	230.0 ± 9.7	39.6 ± 3.4	100
Series 4					
p-Methoxybenzyl analogue	—	—	4610 ± 220	2.09 ± 0.12	5.88 ± 0.39
Allethrin	—	0.11	96.2 ± 2.7	100	282 ± 15
Pyrethrins	3.31	0.21	271 + 12	35.3 + 1.9	100

(44) tested the new esters against house flies and submitted the data given in Table XIII.

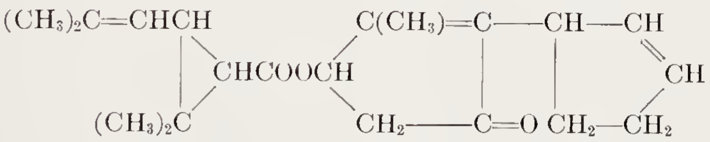
Incho and Ault (71) evaluated the allethrin analogue with a thenyl side chain (XI) as well as furethrin (X) and benzythrin (VII, R = benzyl). No details of preparation or properties of their pyrethroids were given. Using the turntable method and 3-day-old house flies as the test insect, they found that furethrin was 0.58 times as toxic as allethrin, benzythrin 0.47 times, and the thenyl analogue 0.43 times. The response of these pyrethroids to synergism varied from that of allethrin. Furethrin and the thenyl analogue with six or seven synergists was better than allethrin; the benzyl analogue showed little response.



(XI)

Mitsui and Nagase (108) prepared a crystalline phenethrin (m.p. 65–67°C.) and found it to be only 15% as toxic as pyrethrins to mosquito larvae.

In 1954 Haynes and co-workers (67,68) at Boyce Thompson Institute described cyclethrin, a new pyrethroid in which the usual cyclopentenolone ring had a cyclopentenyl side chain (XII). In Peet-Grady tests with house flies they reported that pyrethrins, allethrin, and cyclethrin tested separately gave approximately the same mortality at 24 hours. In mortality studies with piperonyl butoxide and sulfoxide as synergists, 60–80% more cyclethrin was required than pyrethrins to obtain comparable mortality to house flies. Against adult male German cockroaches, with either piperonyl butoxide or sulfoxide, 3 times as much allethrin or cyclethrin was required to obtain mortality on the level of that of pyrethrins and the same synergist. In low pressure aerosols, too, the pyrethrins responded much better to synergism than did either cyclethrin or alle-



(XII)

TABLE XIV
Effectiveness of Unsynergized Pyrethrins, Cyclethrin, and Allethrin for Kill of Three Agricultural Insect Pests (68)
(Concentration required for LD_{95}, in mg./100 ml. of water)

Insecticide	Bean aphids	Spider mite nymphs and adults	Mexican bean beetle larvae
Pyrethrins	3.5	92	32
Cyclethrin	25	290	66
Allethrin	52	270	52

thrin. The authors also demonstrated that these new pyrethroids were less effective than pyrethrins against bean aphids, spider mite nymphs, and adults, and Mexican bean beetle larvae, as shown in Table XIV.

Haynes *et al.* also compared allethrin and cyclethrin with and without DDT in low pressure aerosols using synergists (Table XV).

TABLE XV
Effectiveness of Cyclethrin and Allethrin with Synergists in Presence and Absence of DDT in Low Pressure Aerosols against House Flies[a] (68)

Formulation[a]	No. of tests	Kill in 24 hrs., %	Knockdown in 15 min., %	OTA difference[b]	
				Kill	Knockdown
DDT + cyclethrin + piperonyl butoxide	19	88	81	− 4	+13
DDT + allethrin + piperonyl butoxide	19	92	80	0	+12
Cyclethrin + piperonyl butoxide	11	80	89	−12	+13
Allethrin + piperonyl butoxide	11	61	81	−31	+ 5
DDT + cyclethrin + sulfoxide	19	90	82	− 2	+14
DDT + allethrin + sulfoxide	19	91	81	− 1	+13
Cyclethrin + sulfoxide	11	81	90	−11	+14
Allethrin + sulfoxide	11	68	82	−24	+ 6

[a] Ingredients, in per cent by weight: DDT, 2; cyclethrin or allethrin, 0.34; synergist, 0.6; Freon-11, 42.5; Freon-12, 42.5; methylated naphthalenes, 6.5; made up to 100 with refined petroleum distillate.

[b] For OTA (Official Test Aerosol) difference, figures represent averages of 24 tests for formulations containing 2% DDT and 16 tests for formulations with no DDT.

TABLE XVI
Mortality and Knockdown of House Flies Caused by Kerosene Sprays Containing Cyclethrin, Allethrin, or Pyrethrins, Alone and with Piperonyl Butoxide or Sulfoxide[a] (48)

	Concentration, mg. / dl.			Knockdown in 25 min., %	Mortality in 1 day, %
Material	Insecticide	Synergist	Insecticide equivalent		
Cyclethrin	300			100	81.4
	200			100	58.1
	133.3			100	42.4
	88.9			100	25.0
	59.3			99.5	12.7
Allethrin	200			100	88.1
	133.3			100	70.4
	88.9			100	49.3
	59.3			100	22.5
	39.5			100	8.9
Pyrethrins	759			100	89.0
	506			100	81.1
	338			100	65.2
	225			100	50.9
	150			100	37.8
	100			100	15.7
Cyclethrin + piperonyl butoxide	26.3	263	31.3	100	92.6
	17.6	176	20.9	99.7	58.3
	11.7	117	13.9	96.4	20.8
	7.80	78.0	9.29	89.7	6.0
Allethrin + piperonyl butoxide	59.3	593	66.0	100	95.3
	39.5	395	44.0	100	89.1
	26.3	263	29.3	98.8	50.6
	17.6	176	19.6	94.0	14.5
Cyclethrin + sulfoxide	26.3	263	29.6	100	89.1
	17.6	176	19.8	99.7	65.8
	11.7	117	13.2	97.6	20.3
Allethrin + sulfoxide	39.5	395	42.4	100	87.4
	26.3	263	28.3	99.7	50.6
	17.6	176	18.9	97.5	11.3
Pyrethrins + sulfoxide	12.5	125	15.0	100	74.7
	8.84	88.4	10.6	100	43.3
	6.25	62.5	7.49	100	15.5

[a] Means of six replicates.

It is unfortunate that pyrethrins were left out of this last comparison, since it has been demonstrated that the pyrethroids and pyrethrins do not show the same response to synergism.

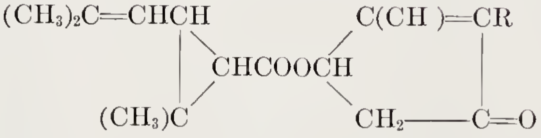
Gersdorff and Piquett (48) compared cyclethrin, allethrin, and pyrethrins with and without the synergists piperonyl butoxide or sulfoxide. Their results are given in Table XVI.

Granett and Haynes (56) tested cyclethrin and pyrethrins with and without synergists and additives as livestock sprays, but none of their mixtures showed exceptional control or repellency.

Blum (12,13) studied the insecticidal effect of eight pyrethroids with and without synergists applied topically to female house flies. Some of the data obtained are compiled in Table XVII. Clearly, all the pyrethroids tested by Blum are subject to synergism, although the degree of synergism varies in the series. He also found allethrin without synergist to be about twice as toxic as pyrethrins, whereas

TABLE XVII

Toxicity to House Flies of Pyrethroids in Presence and Absence of Piperonyl Butoxide (13)



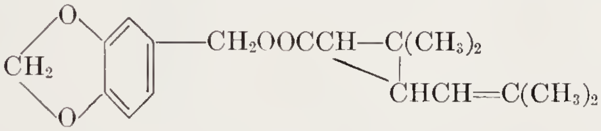
(VII)

R in formula VII	LD$_{50}$ at 24 hrs., μg. Pyrethroid	Pyrethroid + synergist (1:10)
Allyl	0.37	0.165
Methylallyl	0.69	0.143
2-Chloroallyl	1.60	0.33
3-Chloroallyl	1.81	0.22
Cyclopentenyl	0.87	0.13
2-Furfuryl	1.52	0.32
Thiophenyl	1.54	0.20
Phenyl	2.96	1.00
Benzyl	1.20	0.23
1,3-Pentadienyl 2-Butenyl } pyrethrins	0.68	0.089

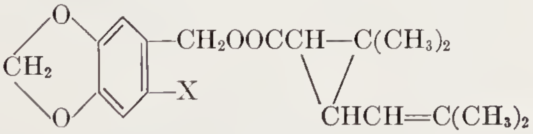
with the synergist, the pyrethrins were about twice as effective as allethrin. The heterocyclic analogues were less toxic and responded less to synergism than the allylic or allyl side chain analogues. The benzyl analogue was more toxic and responded more to synergism than the phenyl. Addition of methyl to the side chain of the allyl analogue lowered the toxicity as did chlorine.

V. Benzyl Chrysanthemumates

Staudinger and co-workers (133,135,136) were the first to study the insecticidal effect of esters of natural chrysanthemumic acid with alcohols other than cyclopentenelones. Of this class of esters, they reported none showing promise as an insecticide. Later Harvill (66), Synerholm (139), Inoue (73), Mitsui (106–108), Barthel (8,20), and Gersdorff (51–53,105,121), and their co-workers, studied similar esters of chrysanthemumic acid. One of the early promising leads was piperonyl chrysanthemumate (XIII), first prepared by Staudinger and Ruzicka (136) and 25 years later patented as an insecticide by Synerholm (139). This ester was also considered by Inoue (73) and by Chen and Barthel (20). Barthel and associates (7,9) continued the study of related esters which finally led to the preparation of 6-bromopiperonyl (XIV, R = Br) and 6-chloropiperonyl (XIV, R = Cl (barthrin)) chrysanthemumates (7). A comparison of the toxicity to house flies of the unsubstituted piperonyl ester with the halogenated piperonylesters is given in Table XVIII.



(XIII)
Piperonyl chrysanthemumate (ENT 20274)



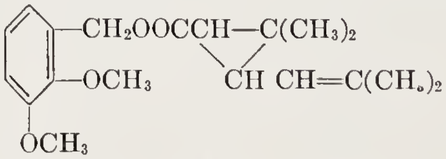
(XIV)
Barthrin: X = Cl

At the time the substituted piperonyl chrysanthemumates were being studied, other substituted benzyl chrysanthemumates

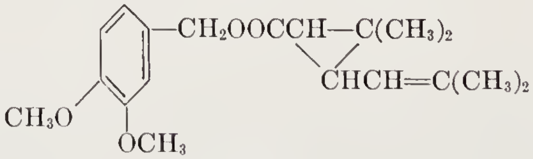
TABLE XVIII
Toxicity of Piperonyl Chrysanthemumates to House Flies (10)

Compound	Concentration, mg./ml.	Mortality in 1 day, %	LC_{50}, mg./ml.
Piperonyl chrysanthemumate (ENT 20274)	13	93	
	8.9	70	
	5.9	31	7
	4.0	9	
	2.6		
		1	
6-Chloropiperonyl chrysanthemumate (ENT 21557, barthrin)	6.7	100	
	4.4	97	
	3.0	79	
	2.0	52	2
	1.3	17	
	0.9	4	
6-Bromopiperonyl chrysanthemumate	10	98	
	6.8	90	
	4.5	67	4
	3.0	32	
	2.0	11	
	1.3	3	

were being prepared and tested. Chen and Barthel (20) showed that very slight changes in substituents produced marked effects on biological activity of the benzyl chrysanthemumates. Especially interesting was the observation that *o*-veratryl (2,3-dimethoxybenzyl) chrysanthemumate (XV) was highly effective against



(XV)
o-Veratryl chrysanthemumate



(XVI)
Veratryl chrysanthemumate

body lice and mosquito larvae, but the isomeric veratryl (3,4-dimethoxybenzyl) chrysanthemumate (XVI) was almost without effect against these two insects.

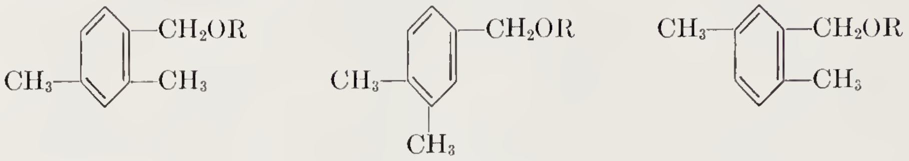
Even more striking was finding in later work (6,9,121) that 2,4-

TABLE XIX
Comparison of Toxicity of Benzyl Chrysanthemumates to Various Insects (6,130)

Concentration p.p.m.	2,4-Dimethyl	3,4-Dimethyl	2,5-Dimethyl	Un-substituted
Mosquito Larvae				
1.0	100	100	100	
0.1	98	99	58	12
0.05	70	88		
0.025	56	84		
0.005	70			
Salt-Marsh Caterpillar				
1.0	100		100	6
0.5	100		100	
0.25	100		50	
0.1	100			
0.05		100		
0.01		65		
Boll Weevil				
1.0	100		5	6
0.5	70			6
Southern Armyworm				
1.0		100		60
0.5			55	
0.1	100	95		
0.05	100	20		
0.01	30			
Body Louse				
[a]	Effective more than 31 days		Ineffective on initial exposure	
House Fly				
20 mg./ml.	100	90	40	
10 mg./ml.	87			

[a] 1% solution on cloth.

and 3,4-dimethylbenzyl chrysanthemumates (XVII and XVIII) were highly effective against lice, mosquito larvae, and house flies, but that 2,5-dimethylbenzyl chrysanthemumate (XIX) had little efficacy. Benzyl chrysanthemumate, the unsubstituted parent compound, had practically no insecticidal properties. A comparison of these four compounds is given in Table XIX.



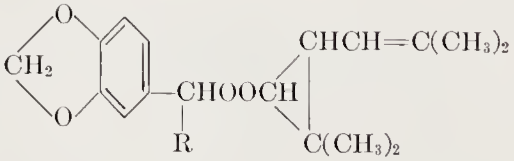
(XVII) ENT 21170 (XVIII) ENT 21825 (XIX) ENT 21563

Dimethylbenzyl chrysanthemumates: R = chrysanthemumyl

Perhaps the characteristic that makes the benzyl chrysanthemumates of so much interest is their low mammalian toxicity. Aldridge and Waring (2) report on work with barthrin and the 6-bromo derivative: "A maximum dose of 4 g. per kg. of body weight administered orally to white mice was considered nontoxic. The dermatology tests were conducted on shaved white rabbits and no trace of irritation of the skin was observed." Ambrose (3) reported the LD_{50} against rats with barthrin to be greater than 15 g. per kg. of body weight.

Because of the ease of preparation and low mammalian toxicity of these new compounds, they are currently being considered as substitutes for some of the chlorinated hydrocarbons in mosquito control work where insecticide resistance has become a problem.

Study of the α-substituted piperonyl esters of chrysanthemumic acid (XX) yielded much biological data (20,89) as shown in Table XX, but the difficulty of preparing these esters seems to rule out commercial interest in them. It is of interest that esters with cyclic substituents showed little toxic action, but were excellent synergists.



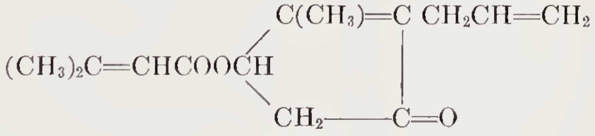
(XX)

King (89) reported the results of tests on body lice, mosquito larvae, fleas, ticks, and roaches at Orlando, Florida, with 52 esters of chrysan-

TABLE XX
Biological Action of α-Substituted Piperonyl Esters of Chrysanthemumic Acid (89)

R in formula XX	Biological action
Methyl	Mosquito larvacide; body louse toxicant and synergist
Ethyl	Mosquito larvacide; body louse synergist
Propyl	Mosquito larvacide; body louse synergist
Allyl	Mosquito larvacide; body louse synergist
Isopropyl	Mosquito larvacide; body louse synergist
Butyl	Mosquito larvacide; body louse synergist
sec-Butyl	Mosquito larvacide; chigger toxicant; body louse synergist
tert-Butyl	Mosquito larvacide; body louse toxicant and synergist
Amyl	Mosquito larvacide; body louse synergist
tert-Amyl	Mosquito larvacide; body louse toxicant and synergist
Methallyl	Mosquito larvacide; body louse synergist
Cyclohexyl	Body louse synergist
Phenyl	Mosquito larvacide, body louse synergist
Benzyl	Body louse synergist; house fly synergist
Phenylethyl	Body louse synergist; house fly synergist
Phenylpropyl	Body louse synergist

themumic acid. Allethrin, isomers of allethrin, furethrin, benzythrin, and phenethrin were the only pyrethroids in this group that seemed to show much promise, although certain of the α-substituted piperonyl chrysanthemumates had considerable efficacy against body lice. In the same work King also tabulated the results of tests against some of these insects with a few esters of allethrolone with acids other than the cyclopropane carboxylic. Only the senecioic acid ester of allethrolone (XXI) had much effectiveness and this was against body lice.



(XXI)

Senicioic acid, allethronyl ester

Staudinger and Ruzicka (136) prepared 32 esters of pyrethrolone with acids other than the chrysanthemumic acids, but none approached the pyrethrins in toxicity. Since their esters were not purified or assayed for chemical identity, it may be that some of their tests were with materials that had degraded because of the poor stability of pyrethrolone.

Mitsui *et al.* (106–108) prepared about 100 esters of chrysanthemumic acid, including piperonyl chrysanthemumate, but report poor effectiveness against mosquito larvae in comparison with pyrethrins.

Wilson and Labrecque (142), using allethrin and pyrethrins synergized with piperonyl butoxide as a standard, compared Diazinon,* Dipterex,** ronnel (Korlan***), and malathion with five esters of chrysanthemumic acid: 6-chloropiperonyl, 6-bromopiperonyl, *m*-isopropoxybenzyl, *m*-(2-methallyloxy)-benzyl, and 2,4-dimethylbenzyl. They conducted tests in the laboratory, using a wind tunnel and spray chamber, and a practical field test in dairy barns. Although these materials all gave satisfactory 24 hour mortality rates, none significantly reduced the barn population of house flies (*Musca domestica*) over a period of 4 days.

Nelson and Gaham (116), in a comparison using adult salt-marsh mosquitoes as a test insect, report the following:

> Kerosene solutions of 14 experimental insecticides and a malathion standard were tested in the laboratory as contact sprays against adults of mixed species of DDT-resistant salt-marsh mosquitoes, *Aedes taeniorhynchus* (Wied.) and *Aedes sollicitans* (Wlk.) (Culicidae: Diptera).
>
> The ratios of the computed LC_{90}'s to that of malathion were: parathion, 4.92; paraoxon, 4.29; EPN, 1.82; Co-Ral** (Bayer 21/199), 1.13; Potasan**** and Diazinon, 0.64; *O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate (Am. Cyanamid 12008), 0.60; the 6-chloropiperonyl and 6-bromopiperonyl esters of chrysanthemumic acid, 0.38 and 0.31; BHC and Dipterex, 0.14; ronnel (Korlan), 0.05; dicapthon, 0.06; and DDT, 0.03.

Weidhaas, Gahan, and Ford (141), in a study of the factors effecting loss of toxicity of insecticides following application for control of mosquito larvae, found that 2,4-dimethylbenzyl chrysanthemumate and its analogue prepared from hydrogenated chrysanthemumic acid (XXII) did not show loss of toxicity by absorption on the soil as did parathion, malathion, Dipterex, Diazinon, and 13 other insecticides, including piperonyl chrysanthemumate and its 6-chloro and

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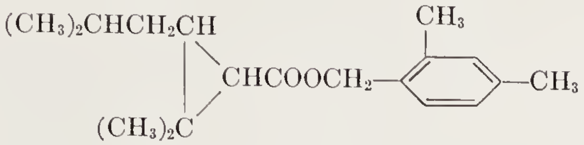
* Registered trademark Geigy Chemical Corp., Ardsley, N. Y.

** Registered trademark of Chemagro Corp., Kansas City, Mo.

*** Registered trademark of The Dow Chemical Co., Midland, Mich.

**** Registered trademark of Farbenfabriken Bayer, Leverkusen, Germany.

6-bromo analogues. Absorption by the soil may be a factor in mosquito control work.



(XXII)

Gersdorff, Freeman, and Piquett (38) studied the difference in toxicity to house flies of the *d-trans-*, the *dl-trans-*, and the *dl-cis*-chrysanthemumic esters of 6-chloropiperonyl alcohol and compared them with the *dl-cis,trans* isomer (barthrin) and allethrin as standards. Results of this study are given in Table XXI.

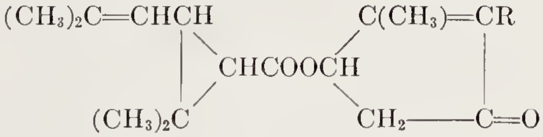
TABLE XXI

Toxicity Relative to Allethrin of Barthrin and Three of Its Isomers (38)

Material	LC_{50}, mg./ml.	Relative toxicity (allethrin = 100)	Range of toxicity ratios between 5 and 95% mortality levels
Barthrin isomers			
d-trans	148.4 ± 4.6	61.86 ± 2.80	45.4–84.3
dl-trans	242.4 ± 7.7	37.87 ± 1.72	27.8–51.6
dl-cis	312.2 ± 9.5	29.40 ± 1.31	21.6–40.1
Barthrin			
Sample B	285.1 ± 8.7	32.20 ± 1.44	23.6–43.9
Sample A	298.1 ± 9.3	30.80 ± 1.39	22.6–42.0
Allethrin	91.80 ± 3.0	100	100

Gersdorff and Piquett (52,53) have carried out extensive studies comparing the various benzyl chrysanthemumates with allethrin and pyrethrins.

Harper, Reed, and Thompson (64) prepared nine pyrethroids which they called DDT-pyrethrin hybrids. These candidate insecticides



(XXIII)

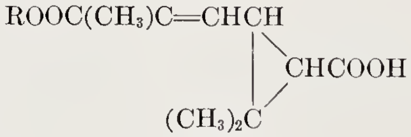
are listed in Table XXII. Only the *p*-chlorobenzyl esters showed any pronounced activity against house flies.

TABLE XXII
DDT-Pyrethrin Hybrids (64)

R in formula XXIII	Chrysanthemumic acid
p-Chlorophenyl	*dl-cis*
p-Chlorobenzyl	*dl-cis*
p-Chlorophenazyl	*dl-cis*
2,2,2-Trichloroethyl	*dl-cis*
p-Chlorophenyl	*dl-trans*
p-Chlorobenzyl	*dl-trans*
p-Chlorophenethyl	*dl-trans*
p-Chlorophenazyl	*dl-trans*
2,2,2-Trichloroethyl	*dl-trans*

VI. Chrysanthemumdicarboxylic Acid

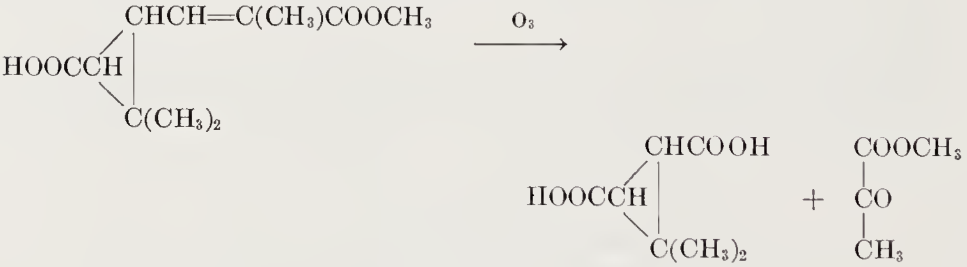
Many hundreds of derivatives have been prepared from chrysanthemumic acid, and their insecticidal properties studied. Yet, except for the original work of Staudinger and Ruzika (134) there has been no corresponding study of chrysanthemumdicarboxylic acid (XXIV, R = H), the acid fragment of pyrethrum II and cinerin II. This



(XXIV)

Chrysanthemumdicarboxylic acid: R=H Pyrethric acid: R=CH_3

is not an oversight, but rather reflects the great difficulty in preparing a synthetic compound subject to a high degree of isomerism because of the lack of symmetry in the molecule.



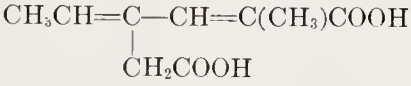
(XXV)
Caronic acid

The configuration around the cyclopropane ring had been shown to be the same as chrysanthemumic acid because it yielded *laevo-trans*-caronic acid (XXV) on ozonization (134). The configuration of the side chain was still in doubt and attempts to elucidate it were hindered because the double bond resisted hydrogenation and bromine added only very slowly.

In 1955 Inoue and co-workers (79,83) prepared *dl-trans*-chrysanthemumdicarboxylic acid from ethyl *trans*-α,δ-dimethylsorbate by addition of ethyl diazoacetate and subsequent hydrolysis. This work established that the side chain of chrysanthemumdicarboxylic acid was *trans*. There was no report on biological data with this material or any of its derivatives.

Harper *et al.* (63,65) reported on the addition of diazoacetic ester to methyl esters of sorbic acids in which they show that there was some inversion of the acids produced. The infrared spectra (65) for the adduct of α,δ-dimethylsorbic acid and the natural chrysanthemumdicarboxylic acid were identical.

Inoue *et al.* (85) showed that one of the products of the preparation of the cyclopropanedicarboxylic acid is α,δ-dimethyl-γ-carboxymethylsorbic acid (XXVI).

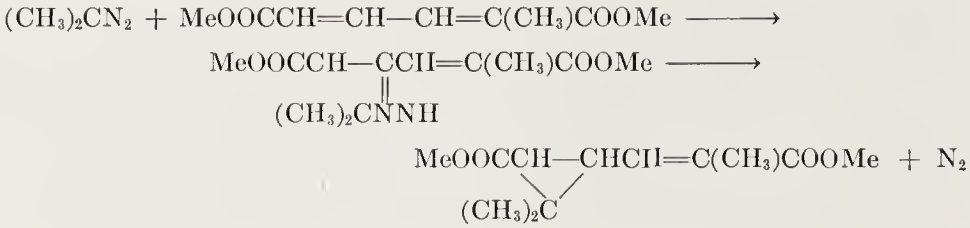


$$CH_3CH{=}\underset{\substack{|\\ CH_2COOH}}{C}{-}CH{=}C(CH_3)COOH$$

(XXVI)

Inoue and co-workers in an exhaustive study (72,75–78,80,84) reported on the unequivocal synthesis of the chrysanthemumdicarboxylic acid identical with the natural material. They showed this to be the *d-trans*-3-(*trans*-2-carboxypropenyl)-2,2-dimethylcyclopropane-1-carboxylic acid. In this work Inoue and Ohno (78) resolved the synthetic (±)-*trans,trans*-chrysanthemumdicarboxylic acid into its optical isomers by means of the phenylethylamine salt.

But Inoue and his colleagues did not stop with the synthesis of the natural material; they went on to devise a new synthesis by which the (±)-*trans,cis*-chrysanthemumdicarboxylic acid could be prepared (81,82,138,140). Instead of using the conventional addition of ethyl diazoacetate to a substituted sorbic acid, they added dimethyldiazomethane to the dimethyl α-methylmuconate of proper configuration as follows:

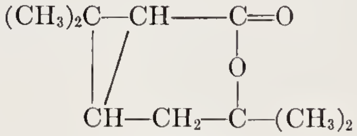


Unfortunately, in none of these works has there been reported a biological evaluation of the acids or their derivatives.

VII. Pyrocin

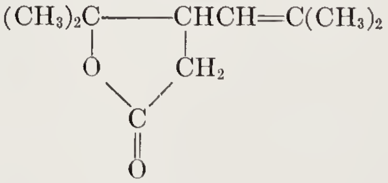
There is one class of compounds which must be considered with synthetic pyrethroids, although they are actually degradation products of pyrethrins.

Matsui and Nagase (100,114), in a study of insecticidal constituents of the smoke of pyrethrum flowers, isolated a compound (XXVII) to which was given the structure 1,1-dimethyl-2-(α-hydroxy-α-methylpropyl)cyclopropanecarboxylic acid lactone and the name pyrocin. Matsui attributed a considerable insecticide action to this compound. He was able to synthesize it by heating chrysanthemumic acid in a sealed tube.



(XXVII)
Pyrocin

Crombie, Harper, and Thompson (24) challenged this structure and in a rather extensive study advanced an alternate based on the scission of the cyclopropane ring (II). Further, these authors claimed that this material was an artifact which had no insecticidal action.



(XXVIII)

It is difficult to resolve these opposite views, but it would appear that further study of this and related compounds is in order.

VIII. Relationship between Structure and Activity

Perhaps one of the most noticeable and yet discouraging aspects of synthetic pyrethroids has been the lack of correlation between structure and activity. This should probably not be surprising in view of the fact that even among the geometric isomers of allethrin there can be a 100-fold difference in activity against house flies.

Part of this lack of correlation can be traced to incomplete studies of individual series. It is apparent that β-unsaturation in a straight side chain is present in both allethrin and cinerin I, compounds of high activity. Yet only one other β-unsaturated straight chain analogue was made—the 2-pentenyl compound of Crombie *et al.* (23)—even though its activity was reported as promising; obviously, others in this series should be made. The 2-methallyl analogue (37,73, 88,126) of allethrin was also promising, but no further study of related branched chain compounds was reported.

Only nine different analogues of cyclopropane acids were reported in the study by LaForge *et al.* (94) of variations in the acid component. In view of the hundreds of esters of chrysanthemumic acid that have been prepared, the study of the variations of this acid has been woefully neglected.

In general, one may make a few rough conclusions. Among allethrin analogues a straight β-unsaturated side chain is most active. Saturated side chains decrease activity from methyl through ethyl and propyl; the butyl and amyl analogues are inactive. *trans*-Chrysanthemumic acid esters with *d*-configuration tend to be more active than those isomers of opposite arrangement.

Among simple esters of chrysanthemumic acid, the aromatic esters show the highest activity. The 6-chloropiperonyl chrysanthemumate is the most effective of the benzyl chrysanthemumates. Next in line, and about equally toxic, are the 6-bromopiperonyl and the 2,4-dimethylbenzyl and 3,4-dimethylbenzyl chrysanthemumates. Substitution at the α-position of the benzyl alcohol almost destroys toxicity of the ester to house flies. Also, even one more or one less methylene group between the ring and the ester linkage destroys fly toxicant action—the phenyl and phenylethyl derivatives seem without promise as toxicants from data so far presented.

IX. Summary

One of the most significant developments in the field of organic insecticides has been that of synthetic pyrethroids. When all other classes of insecticides studied seem to be of a relatively high order of mammalian toxicity, the synthetic pyrethroids exhibit extremely low mammalian toxicity. Of the hundreds of synthetic pyrethroids prepared, many have shown promise. Three—allethrin, cyclethrin, and furethrin—went into commercial production, and three others—barthrin and the 2,4 and 3,4-dimethylbenzyl chrysanthemumates—are now at the pilot plant stage. Perhaps this has more significance than is at first apparent. With insects rapidly becoming resistant to the chlorinated hydrocarbons and the phosphates, there may soon be a desperate need for insecticides to replace those no longer effective.

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SELECTIVE TOXICITY OF INSECTICIDES*

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I. Introduction

Why do we want selective pesticides? For two reasons: to reduce hazard, and to avoid unfavorable changes in the biological complex into which we are introducing the poison.

Hazard in modern pesticides is probably at its worst with the organophosphates, even though several are extremely safe. Metcalf (44) estimates that the most used phosphorus insecticides are parathion and methyl parathion, of which 20,000,000 lb. are made annually. Much of this dangerous material is handled by, shall we say, very unsophisticated people. Fortunately, most of them follow the label instructions well enough, and cases of fatal poisoning are surprisingly rare. But reported poisonings are high, e.g., 25 in Florida in 1950 (26), 103 in California in 1954 (10) (both from parathion alone), and undoubtedly many more cases go unreported. One may expect higher accident rates in countries where the workers are less educated or facilities for decontamination are poorer, and Hayes (27a) reports that Japan has over 300 deaths annually from parathion alone. Barnes (6) has reviewed the hazard problem excellently, so it will suffice to state here the belief that universities, governments and industries have an urgent responsibility to ensure that in a reasonable time (say ten years) there are enough good low-hazard insecticides available to make it possible to outlaw the use of any hazardous organophosphates. Every death, no matter how carelessly caused, is a reproach to our abilities as scientists and organizers.

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The problem of the unfavorable changes in the biological complex caused by using nonselective insecticides has been splendidly discussed in a review by Ripper (73), from which one sentence is particularly choice: "Where paraoxon was used commercially it produced on the sprayed fields the most enormous cabbage aphid outbreak within a fortnight after spraying which has ever been seen in England. . . ." There is some conflict on this problem however: although the control entomologists often want selective insecticides, the manufacturers commonly want wide-spectrum insecticides, so that they may have a large market and can cut down on the research and fees needed for registering pesticides. Scientifically, however, it is our business to provide selective pesticides, and it is now time to discuss how we can achieve this end.

II. Principles

The basic problem of selective toxicology is to find out how to kill enemies and spare friends. The enemy may be an insect, mammal, fungus, virus, bacterium, weed, protozoon, etc. The friend will usually be man, his domestic animals and plants, and those smaller organisms which are useful themselves (e.g., bees) or parasitic or predatory upon inimical organisms. In chemotherapy one may have in addition the requirement to kill one kind of tissue (i.e., neoplastic) and spare another in the same animal. The first part of this work will deal with some of the principles applicable to the immensely diverse problems in which both friend and enemy are animals. We shall then consider the achievements and proposals with emphasis on the organophosphates; both selective insecticides and selective mammalicides will be considered.

In the following pages *the enemy* will refer to the species which one wants to kill, *the friend* to the species which one wants to spare. These terms will also be used collectively, so that *the friend* can imply, say, livestock, domestic pets, and man. This proposal admittedly contains the seeds of some ambiguity, but the context will be arranged in each case in order to make the matter clear.

When an animal is poisoned, a complex set of events occurs. We may classify these into five major phases:

1. Arrival in the body: application and penetration.
2. Metabolism: activation and degradation.
3. Disposal: excretion and storage.
4. Penetration to the target area.
5. Attack on the target: attachment, reaction, reversal.

This set of events is only a part of the whole history of the poisoning; and can be called "the development of the critical biochemical lesion." (The term *biochemical lesion* was introduced in 1931 by Sir Rudolph Peters (25,66) to explain his then novel concept of gross effects being attributable to specific biochemical disturbances.) When the critical lesion is established, further biochemical and physiological events occur which cause the symptoms or death. Thus in anticholinesterases the biochemical lesion is the inhibition of cholinesterase (at any rate in the mammal), and this in turn leads to respiratory paralysis, either by central or peripheral effects, and thence to death by asphyxia. This second set of events can be called "consequences of the biochemical lesion."

The use of the term *biochemical lesion* does not mean to imply that all poisons act on enzymes: DDT may well act by being built into a crucial part of a nerve membrane, thus disrupting it. The term is used simply to point out that the fundamental disturbance is at the molecular level. The term critical is used here as a reminder that other noncritical biochemical lesions will very probably occur: for instance, chymotrypsin will very probably be inhibited in a mammal poisoned by organophosphates. We cannot, of course, exclude the possibility that the consequences of a noncritical lesion may accentuate the effects of a critical lesion, but at present there is little evidence to suggest such an interaction.

If this account just given is a complete one (and it is supposed to be), then we can enunciate the first principle: a difference in toxicity between two species can only be due to a difference in either (*a*) the five phases involved in development of the critical lesion or (*b*) the consequences of that lesion.

Now, as to the consequences of a given critical lesion we can usually do very little, although perhaps if the consequences depend in part upon environmental factors we might arrange the environment (e.g., by choosing a particular climatic condition) to favor recovery of the friend and death of the enemy. Such an approach is useless at present because we know so little about the lesions' consequences. For instance, although it is virtually certain that when the biochemical lesion is cholinesterase inhibition, the critical consequences in the mammal are inhibition of respiration (centrally or peripherally) and consequent death by asphyxia (5,18,19), we have no idea of what are the critical consequences for the insect. Very probably the nervous system is disrupted, but then what happens? Brief experiments (38) have indicated that in the American cockroach death is not due to desiccation. This aspect urgently needs study.

When we consider the development of the lesion, however, we find that there are ways of modifying the pesticide to produce the lesion in the enemy but not the friend. We will now consider how, in principle, this can be done, and then give practical examples.

If we consider any given class of compounds which all attack the same target, i.e., all induce the same critical lesion, then any selective toxicity must be due to a difference in one of the five phases in the development of the lesion listed on page 77. The approach advocated is (*a*) to study the known selective compounds and establish where the differences lie which confer their selectivity and (*b*) to use these findings to incorporate selectivity into molecules whose other properties are desirable. I am afraid I have to introduce here an unpleasant Greco-Roman neologism *selectophore*, which will be the term applied to a group such that its introduction into a toxic molecule confers selectivity upon that molecule. There is, of course, no reason to expect that this will always be feasible; when the overall physical properties of the molecule determine its behavior in a given stage, it may be difficult to arrange that a particular substituent can produce a desired effect. But when a metabolic step is involved, addition of a single group (or even atom) in the right place is very likely to block or promote a reaction, and the possibility of a selectophore occurs.

III. Practice

A. DIFFERENCES IN ARRIVAL IN THE BODY

In spite of identical intrinsic toxicities, a compound will kill the enemy and not the friend if it can be applied in such a way as to cover the enemy more fully, or if it penetrates more readily into the body of the enemy than the friend.

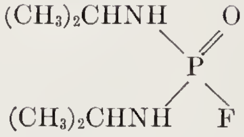
1. Application

This is perhaps the oldest way of obtaining selectivity. In its simplest version, the application technique is designed so that the insects are sprayed with poison while the operator is not. There are numerous other devices—respirators and protective clothing for operators, and so on, but these are obviously matters of common sense. This aspect deserves comment only for completeness' sake, with perhaps the reminder that it is upon this that one falls back when more scientific selectivity is not offered. Most cases of accidental poisoning represent inadequate attempts to obtain application selectivity.

In 1951 Ripper *et al.* (75) proposed the term *ecological selectivity* to describe those cases where selectivity is based upon differences in the ecology (i.e., habitat and feeding habits) of pest and beneficial insects. These authors also proposed the term *physiological selectivity* to describe cases where physiological differences cause selective toxicity; this phrase embraces all the kinds of selectivity dealt with in this paper with the exception of that obtained by special application techniques.

Plant-systemic action offers the possibility of obtaining ecological selectivity: once the insecticide is in the plant, only phytophagous insects will consume it directly. However, parasites and predators may also be poisoned. First, there may be difficulties in avoiding contact with the parasites and predators while applying the compound. Second, unless there is some physiological selectivity as well, parasites and predators may be poisoned by consuming the affected pests, but we may hope that some degradation will have occurred in the pest and also that the parasite or predator will not be consuming poison in such a concentrated form as did the pest.

Ripper *et al.* (75) found that mipafox (I) had no physiological selectivity, so that in spite of its systemic property there was a considerable loss of parasites and predators if the compound was sprayed onto cabbages infested with aphids. However, if mipafox was applied via the soil or the irrigation water, only the pests were killed. Clearly, systemic activity alone (i.e., without physiological selectivity) can confer selective toxicity.



(I)

A device that utilized differences in feeding habits was employed by Ripper *et al.* (74) to make DDT selective. By coating the DDT particles with a degraded cellulose preparation, contact toxicity was eliminated. Those insects which consumed the "pills" along with the plant could digest off the cellulose and poison themselves; the others escaped. This is an ingenious way of taking advantage of ecological selectivity even with an unpromising compound. It could be applied to many other compounds, but economic factors appear to forbid it (75).

The replacement of surface by systemic poisons would often reduce the mammalian hazard. Thus D. M. Norris (unpublished) proposes a vigorous search for a systemic insecticide to control the elm bark beetle, which is a vector for Dutch elm disease. The widespread use of DDT for the control of this insect is commonly observed to cause considerable fatalities in birds (robins in particular). Probably the birds are poisoned by consuming worms which have picked up DDT from the soil (the worms may even concentrate the DDT in their bodies). Such by-products of tree protection are bad from both scientific and propaganda viewpoints.

In at least one case selectivity given by systemic use has been wrongly attributed to application selectivity. It was stated that schradan was ineffective by contact action, only becoming effective after absorption by the plant (49,83). This could explain why only sap-sucking insects were affected. Later work, however, has shown that the selective action of schradan is physiological, since it is observed with direct application in the laboratory: the LD_{50} by injection is 175 mg./kg. for the blowfly and 1340 mg./kg. for the American cockroach (nonsusceptible), and 16 mg./kg. for the squash bug and 30 mg./kg. for the milkweed bug (susceptible) (57,59).

It is apparent, then, that selective systemic insecticides may owe their selectivity to physiological rather than ecological selectivity; but it is also probable that, among compounds which lack physiological selectivity, those which are systemic may have a measure of ecological selectivity. For this reason (among several others) systemic action is often a highly desirable property in an insecticide. There is a very serious need for a comprehensive study of the factors responsible for systemic action, a study which should in the first place be quite unconcerned with the toxicity of the compounds. A few years' work might well enable us to predict with some precision whether a projected new compound would have systemic action. Probably a very few physical parameters decide the matter.

2. Absorption

Let us use the following terms to describe routes: *cutaneous* to refer to surface applications both to insects and mammals; *oral* to refer to application via the alimentary canal; *respiratory* to refer to absorption by lung or spiracle; *intraperitoneal* to refer to injection into the body cavity.

We may subdivide Ripper's class of physiological selectivity into two subclasses *absorptive* and *intrinsic.*

In those cases where selectivity is still observed when ecological factors are bypassed, i.e., by applying the poison directly to the animals under comparison, we can say that the selectivity is physiological. Physiological selectivity may be due simply to a failure to get into the body; if so we can call it absorptive selectivity. To decide this, we can compare the selective toxicity obtained by cutaneous, oral, and respiratory routes with that obtained by delivery directly into the body—intraperitoneally or (better still) intravenously. If selectivity is obtained even when the poison is delivered into the body, we may call it intrinsic selectivity. A summary of this classification, and an indication of the kinds of selectivity which may be termed intrinsic, is as follows:

- Selectivity
  - Ecological
  - Physiological
    - Intrinsic
      - Metabolic
        - Degradative
        - Activative
      - Excretory
      - Target
      - Penetrative
    - Absorptive

There will undoubtedly be cases of interaction between these factors. For instance, slow absorption will give low toxicity if there is a degradative system present. This condition has been mimicked by Saunders (81) who administered TEPP (tetraethyl pyrophosphate) and DFP (diisopropyl phosphorofluoridate) intravenously to rabbits in successive small doses over various periods of time. There was a linear relationship between the total dose required to kill the animal and the time over which it was administered. By giving the poison slowly, the degrading enzymes were given an opportunity to show their action. Probably every poison can be degraded or excreted to some extent by every animal; it is the rate of these processes rather than their absolute occurrence which is important. Therefore extremely slow absorption will automatically give protection, and although we are oversimplifying the case, we can say that the protection is "due to" the slow absorption.

Let us now consider absorption effects for particular compounds. The skin of mammals and the integument of arthropods are such vastly different tissues that one would expect very many compounds to penetrate one and not the other. Unfortunately there is very little known about the relation between chemical structure of compounds and their ability to penetrate either tissue.

Table I presents data on DDT poisoning by various routes. The data are by no means strictly comparable, as they are culled from several authors. It is very clear that to the rat and rabbit DDT is almost harmless if given cutaneously, but is fairly toxic if given intravenously. As for insects, the integument presents a barrier in the milkweed bug and bee (comparing cutaneous and intraperitoneal LD_{50}'s) but not in the Japanese beetle or American cockroach. Clearly a study is needed to examine this interesting variation of integument penetrability with insect species. In many cases this variation may account for the selective toxicity of contact sprays

TABLE I
Influence of Administrative Route on Toxicity of DDT to Insects and Mammals[a]
(LD_{50} in mg./kg.)

	Cutaneous	Oral	Intraperitoneal	Intravenous
Rat	3000	400	150	50
Rabbit	300–2820	300	2100	50
Milkweed bug	409	301	31	
Bee	114	1.7	0.2	
Japanese beetle	93	205	162	
American cockroach	10		7	

[a] Data approximated from Negherbon (49).

Most compounds are more poisonous orally than cutaneously. (In general, toxicity increases with route thus: cutaneous, subcutaneous, oral, intraperitoneal, intravenous. For the respiratory route it is difficult to obtain comparable figures, i.e., in mg./kg. If one could, the LD_{50} would probably be similar to that for the intraperitoneal route.) In the case of DDT the oral toxicity to mammals is still fairly low; but to the bee DDT is very toxic orally, in spite of the low cutaneous toxicity. Such a striking difference between oral and cutaneous toxicity is rather unusual in compounds with low water solubility.

In general, then, it is true for DDT that it is selectively toxic to insects as compared to mammals when applied by any but the intravenous route. Its selectivity is therefore absorptive. Dresden has concluded that the same is true for γ-benzene hexachloride (20). For practical purposes absorptive selectivity is amply adequate to prevent hazard, since animals are unlikely to be exposed except by the oral, cutaneous or respiratory routes.

The cyclodiene compounds (aldrin, dieldrin, heptachlor, chlordane, etc.) are somewhat more hazardous than DDT to mammals, but there is still substantial selective toxicity toward insects. Table II gives a sample of the very limited data available. All we can say is that external application shows marked selectivity (best for heptachlor, worst for aldrin). From the data on chlordane on mammals, it seems that the intraperitoneal route about doubles the toxicity (comparing with the oral route) and the intravenous increases it ten times. It is remarkable that more data on the intraperitoneal and intravenous toxicity is not available. We may say (very tentatively) that, for chlordane at least, poor absorption accounts for much of the low toxicity to mammals.

TABLE II
Toxicity of Cyclodiene Compounds (33,43,49,90)
(LD_{50} in mg./kg.)

	Chlordane	Heptachlor	Aldrin	Dieldrin	Toxaphene
House fly[a]	4	2	2	1	11
American cockroach[a]	10	1	1	2	
Rat					
Cutaneous	534	90	67	87	69
Oral			46	38	
Intraperitoneal	200				
Intravenous	30				
Rabbit					
Cutaneous		2000	600	385	900
Oral	300				780

[a] Topical.

In the case of organophosphates, only a little selective toxicity derives from absorptive effects, because these compounds penetrate both the integument of insects and the skin of mammals. However, as Table III shows, in the case of the more hydrophilic compounds such as schradan and DFP, the mammalian skin presents an effective barrier. There is evidence that malathion, which is toxic to the American cockroach, is nontoxic to the German cockroach because of poor penetration of the integument (38). Apologies are offered for the painfully inadequate data, but few workers appear to have been interested in comparing routes.

A particularly fascinating selectivity is that in which one strain of a species is resistant to a pesticide. For example, considerable differences have been found in the penetration of DDT into various strains of house fly, but this factor does not appear to be of importance in determining resistance, since there is no correlation between resistance and DDT permeability in several strains (87,95) and since resistant flies are also resistant to injected DDT (89). Nor did DDT penetrate less readily into resistant than into susceptible boll weevils (7). However, there does appear to be a correlation between resistance to knockdown and slow penetration (64). Oppenoorth (63) has shown that penetration is not an important factor in the resist-

TABLE III
Toxicity of Organophosphates
(LD_{50} in mg./kg.)

	Parathion	Paraoxon	Systox*	Schradan	DFP	DDVP	Dipterex*	EPN
American cockroach								
Topical	1.2	0.8						
Injection	1.0	0.7						
Bee								
Topical	1.5							
Injection	0.9							
Rabbit								
Cutaneous	45		24	780	117	18	50[a]	
Oral				25	7	3		
Intraperitoneal					1		80[a]	
Rat								
Cutaneous	21		14					
Oral	13		6		8		475	40
Intraperitoneal	7		3	8			225	64

[a] LD_{100}.

ance of house flies to parathion, since this resistance is substantially the same with injected as with contact application. In the case of Diazinon**-resistant flies, Krueger *et al.* (39) have shown that these are only 7% less readily penetrated by labeled Diazinon than are normal flies.

3. Conclusion

Absorption selectivity can be enormously important and may, in fact, account for a good deal of the low mammalian hazard of many chlorinated hydrocarbons. Since differential toxicity based on this difference is usually amply adequate for practical purposes, it is surprising that so little attention has been paid to the factors governing absorption. There are obviously some compounds (such as DDT) which penetrate the mammalian skin with great difficulty. The reason for this valuable property should be found, so that it can be used in the design of selective compounds. A study should be made without reference (in the first place) to toxicity. It is quite likely that penetrability is dictated by a relatively small number of physical parameters.

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* Registered trademark Chemagro Corp., Kansas City, Mo.

** Registered trademark Geigy Chemical Corp., Ardsley, N. Y.

B. DIFFERENCES IN METABOLISM

We now consider the first of what were called in the terminology of the last section *intrinsic* differences. It is in the establishment and exploitation of metabolic differences that there is room for the greatest ingenuity, since quite subtle modifications of a molecule may profoundly alter its behavior in biochemical systems.

All of the organic insecticides can be degraded in the bodies of animals; some of them can be activated, i.e., converted to more potent attackers of the target. A good deal is known about these two processes in organophosphates. These will therefore be discussed first, but for present purposes they will be considered above all as models, demonstrating principles that can and should be extended to quite different pesticides.

Let us consider first compounds toxic to insects but not mammals. Most of the strongly selective organophosphates are phosphorothionates, i.e., they contain the P=S group, and they are poor inhibitors of cholinesterase. They are activated by enzymic oxidation to the phosphate analogues (P=S → P=O) in the liver (principally) of mammals, and in several tissues of all the insects so far studied; the phosphate analogues are usually very good inhibitors of cholinesterase. Both the phosphorothionate and the phosphate may be degraded, and the degrading enzymes are almost all hydrolytic.

The possibility that selectivity could be due to rapid degradation in the nonsusceptible species was first proposed by March *et al.* (40) for malathion on the basis of the smaller number of metabolites found in insect than in mammalian and avian preparations. Fuller studies (38) have now substantiated the hypothesis, but have shown that in fact more metabolites are produced in the intact insect than in the intact mouse.

Another approach was used by O'Brien (52): after addition of malathion to insect tissue preparations a steady formation of its phosphate analogue malaoxon was found (as judged by anticholinesterase activity) whereas no such accumulation followed its addition to mouse liver slices, even though such slices are known to oxidize other phosphorothionates. Malaoxon added to similar preparations was degraded rapidly by the liver slices and slowly by the insect preparations. It was concluded that the balance of activating and degrading enzymes in the insect tissues favored malaoxon formation, but that in the liver the balance favored a net degradation.

This crude but informative system has been extended to acethion (60) and Co-Ral* (61). Acethion is toxic to flies, not to mice or American cockroaches; it is activated by minced flies, degraded by mouse liver slices or minced American cockroach. Co-Ral is toxic to mice, not to cows or rats; it is activated by liver slices of mouse but degraded by liver slices of the cow or rat. Yet in the cow the activating enzyme for Co-Ral is present; in this as in all the other cases it is not the presence or absence of an enzyme system which decides toxicity, but the balance of degrading and activating enzymes.

The development by Casida of techniques for preparing labeled organophosphates of very high specific activity (11), and by Plapp and Casida of efficient column chromatographic separatory techniques (69), has made it possible to study the detailed metabolism of these compounds in intact insects as well as mammals. The metabolism of malathion, Diazinon, dimethoate, acethion, and parathion has been studied with these techniques by Krueger and O'Brien (38) and Krueger *et al.* (39), the emphasis being on the correlation between metabolism and differential toxicity. The technique consisted in treating animals with the radioactive compound, sacrificing them at various times thereafter, and analyzing the whole body for various metabolites, in particular for the phosphate analogue.

The toxicity of these compounds is given in Table IV. There are a variety of differences. Some compounds are toxic to insects but not mammals; some are toxic only to certain insects; the selectivity varies from 1 up to 350-fold. The hypothesis that the authors attempted to test was that if selectivity in these compounds was due to metabolic differences, these should result in differences in the production of phosphate analogue (since this is the actual toxicant). Let us first discuss only differences between insects and mice.

The first observations were extremely encouraging: after malathion poisoning the levels of malaoxon were much higher and more persistent in the susceptible American cockroach than in the nonsusceptible mouse (Fig. 1). A marked difference of a similar kind was found

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* Registered trademark Chemagro Corp., Kansas City, Mo.

TABLE IV
Toxicity of Organophosphates (16,38,39,60) (LD_{50} at 24 hrs., mg./kg.)

	Mouse (female)[a]	American cockroach[b]	German cockroach[b]	House fly[b] (DDT-susceptible)
	Phosphorothionates			
Malathion	815	8.4	120	30
Diazinon	40	2		1.5 (60[c])
Acethion	1280	700		9.4
Dimethoate	140	2		0.4
Parathion	5.5	0.9		0.9
	Phosphate Analogues			
Malaoxon	75	1.5	15	15
Acetoxon	214			3.4
Dimethoate phosphate analogue	55 (rat[d])			0.1

[a] Intraperitoneal.
[b] Topical
[c] DDT-resistant house fly.
[d] Oral.

with the other selective compounds also. However, although parathion exhibits a rather small (6-fold) selectivity between insects and mice, it too showed the phenomenon of phosphate levels being far less in the mouse than the insect (Fig. 1). This disconcerting observation made necessary a close inspection of the correlations between metabolite differences and toxicity differences among the house fly, German and American cockroaches, and mouse following treatment with malathion, parathion, acethion, Diazinon, and dimethoate.

The tentative conclusion arrived at (39) were that the strongly selective compounds fall into two classes: (*a*) malathion and Diazinon, which owe their selectivity to the differences in level and persistence of their oxygen analogues in the body and (*b*) acethion and dimethoate, which owe their selectivity to the differences in level and persistence of the total chloroform-extractables in the body (these comprise mainly the unaltered parent compound). Examples of both classes are given in Figure 1.

In support of this unexpected class *b* selectivity are one theoretical and one practical consideration. First, it is very probably true

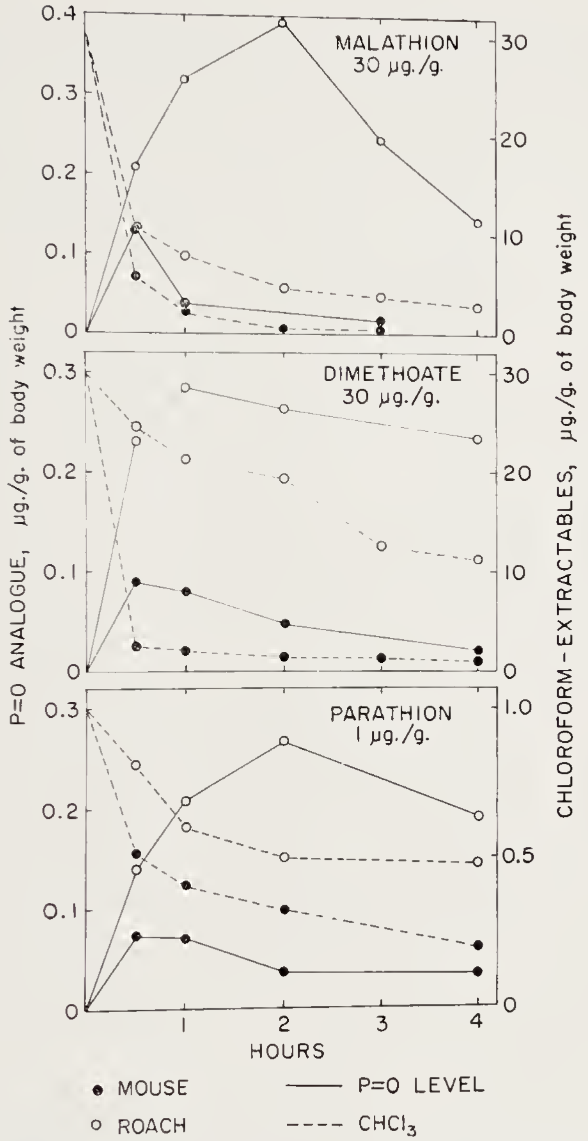


Fig. 1. Levels of P=O analogue and of total chloroform-extractables at various times after injection of mice or American cockroaches with phosphorothionates. Levels expressed in μg. of material (expressed as equivalent phosphorothionate) per gram of body weight. (Data of Krueger and O'Brien, 38; Krueger *et al.*, 39)

that the level of toxicant (i.e., phosphate analogue) *at the target* determines toxicity. But all we have measured is the average level throughout the body. Much of this may be stored away and ineffective, in fat depots for example. The ratio of total toxicant to toxicant at the target will undoubtedly vary with each compound, reflecting variations in capacity to partition into lipoidal from aqueous phases. Second, it has been shown by Seume and O'Brien for the rat (82) that the enhancement of malathion's toxicity induced by EPN is associated *in vitro* with greatly increased levels of malathion, but actually reduced levels of malaoxon; the conclusion was drawn that induced persistence of low levels of malaoxon was responsible for the enhancement of toxicity. Similar conclusions were drawn from *in vivo* studies on the rat by Knaak and O'Brien (35a).

On the toxicity differences between insects (Table IV) the metabolic findings throw practically no light. In spite of the 40-fold resistance of the Diazinon-resistant (compared with normal) house flies, there are only a 2-fold difference in diazoxon level and a 3-fold difference in chloroform-extractables. We are at a loss to say whether or not these are sufficient to explain the resistance. (van Asperen and Oppenoorth (4b) observed that homogenates of resistant house flies degraded paraoxon and diazoxon faster than those of susceptible house flies, and attributed resistance to this difference. However, such an attribution is rather risky with *in vitro* evidence.) With dimethoate the chloroform-extractables are higher in the American cockroach than in the house fly, yet the compound is more toxic to the house fly! It is true that several reasonable differences may be picked out: acethion leads to levels of acetoxon and chloroform-extractable which fall in the expected order for house fly, cockroach, and mouse. But at the moment the position with regard to differences in insect toxicity is definitely obscure, and it is impossible to make any useful statement—except that all such studies must be done with several compounds and several species before one dares make generalizations.

Let us return to a consideration of insects as compared with mammals. The balance of activating and degrading enzymes favors the mammal. Is this primarily because it has better degrading enzymes, or weaker activating enzymes? As Table IV shows, the phosphate analogue of three of the four strongly selective compounds are also strongly selective (even though less so than their parent compounds). It is clear that the mammal must have better degrading enzymes than the insect, since activation in phosphorothionates involves oxidation to the phosphate analogue. In the following discussion we shall consider only degradation differences, because these appear adequate to explain selectivity and they can be evaluated separately, whereas most activating systems cannot be freed from degrading systems. However, the possibility of useful activating differences should be borne in mind.

Let us step aside from the main argument to comment on the fact that phosphorothionates are usually more selective than their oxygen analogues. In those cases where the selective mechanism is degradation, this difference is probably due to an *opportunity factor* (54): phosphorothionates require time for activation before they exert their effect, and this delay gives an opportunity for the degrading enzymes to carry out their lifesaving work.

What are the degrading enzymes which are so much better in the mammal than the insect?

First, some terms require definition: *carboxyesterase* will be used as a nonspecific term to describe any enzyme hydrolyzing RCOOR′ to RCOOH + R′OH. *Amidase* will refer to any enzyme catalyzing carboxyamide hydrolysis, e.g., $RCONR'_2$ to RCOOH + HNR'_2. *Phosphatase* will refer to any enzyme hydrolyzing ester and thioester groups of phosphates and phosphorothioates, e.g., $(RO)_2P(O)SX$ to $(RO)_2P(O)OH$ + XSH; $(RO)_2P(S)OX$ to (RO)(HO)P(S)OX.

In the case of malathion we know for certain that the main selective factor is a high carboxyesterase level in the mammal (38). Thus, of the degradation products of malathion in the mouse, carboxyesterases account for about 70%, phosphatases about 20%; in the house fly, carboxyesterases account for 5–20% phosphatases for 50–70%. In the case of dimethoate it seems likely that it is the high amidase content of mammals which confers selectivity, although in this compound phosphatases play a more important role in degradation than in the case of malathion, accounting for about half of the degradation in rats and cows (16). In the case of acethion, again carboxyesterase activity accounts for its rapid degradation in the mammal (60). But Diazinon (78) and Co-Ral (37) are degraded almost exclusively by phosphatases, and experiments described above suggest that their selective action is attributable to a metabolic effect. Ronnel (70), too, appears to be degraded exclusively by phosphatases, but we have no proof in this case that its selectivity is due to metabolic differences. These results are are summarized in Figure 2.

On the basis of the then rather premature conclusion that the high carboxyesterase activity in mammals accounted for malathion's

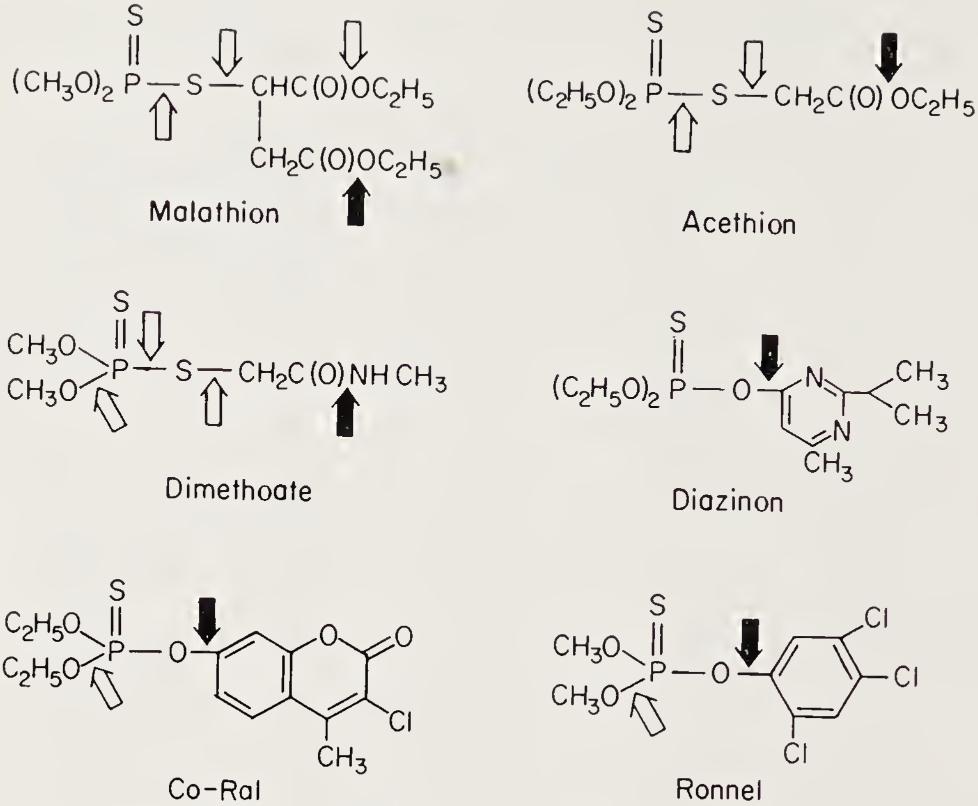


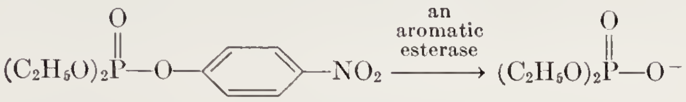
Fig. 2. Sites of enzymic degradation by mammals of organophosphate with low mammalian toxicity. All degradations are hydrolytic; major attacks shown as heavy arrows. (Data of Dauterman *et al.*, 16; Krueger and O'Brien, 38; Krueger *et al.*, 39; O'Brien *et al.*, 60; Plapp and Casida, 70; Robbins *et al.*, 78)

selective toxicity, the following speculation was presented in 1957 (53).

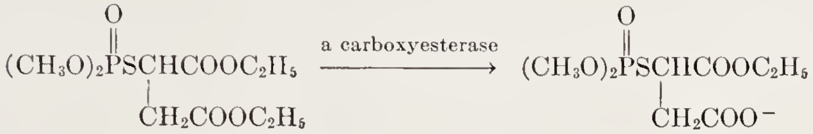
1. It is well known that organophosphates attack cholinesterase by an electrophilic mechanism; i.e., the phosphorus atom has to have a positive character. This positive character is enhanced by neighboring electrophilic (i.e., electron-attracting) groups, and this is why $R_2P(O)X$ compounds are better inhibitors of cholinesterase than $R_2P(S)X$. This topic is admirably expanded by Fukuto and Metcalf (24).

2. It follows that a hydrolysis which leaves an anionic group near the phosphorus will always detoxify, because anionic groups are immensely potent nucleophilic (i.e., electron-supplying) groups, and therefore usually abolish the capacity of the phosphorus to attack

the enzyme. That is why reactions such as equations 1 and 2 are detoxifying.



(1)



$$(CH_3O)_2\overset{\overset{\displaystyle O}{\|}}{P}S\underset{\underset{\displaystyle CH_2COOC_2H_5}{|}}{C}HCOOC_2H_5 \xrightarrow{\text{a carboxyesterase}} (CH_3O)_2\overset{\overset{\displaystyle O}{\|}}{P}S\underset{\underset{\displaystyle CH_2COO^-}{|}}{C}HCOOC_2H_5$$

(2)

3. Malathion is an exotic compound for the animal; it is therefore likely to be hydrolyzed by a carboxyesterase of low specificity. Hence the introduction of a carboxy ester group into any organophosphate should make a compound which the mammal can hydrolyze and thereby detoxify. And since mammals have more of this carboxyesterase than have insects, such a compound should be selectively toxic.

4. Selective toxicity in such a compound should be more evident in phosphorothionates than in phosphates, because of the opportunity factor (page 91).

On the basis of these speculations, several new organophosphates were prepared (60), of which the most interesting was acethion (so called not because it was an ace, but because it was a derived "acetyl phosphorothionate"). These compounds indeed showed selective toxicity as Table V shows. As the ratio L_1/L_2 shows, the compounds were all selective, and the opportunity factor was very evident.

Delight in the outcome of these speculations was somewhat dampened by the knowledge that Phosdrin,* which contains a carboxy ester group, is not selective. It was possible that since Phosdrin is a phosphate the opportunity factor might be lacking. Dr. E. Y. Spencer (unpublished) therefore made the thiono analogue, which we may call thiono-Phosdrin, $(CH_3O)_2P(S)OC(CH_3){=}CHCOOCH_3$. Unfortunately we have been able to obtain so far only the *trans* isomer, and *trans*-Phosdrin is a poor insecticide compared to *cis*-Phosdrin. Nevertheless, preliminary results indicate that *trans*-thiono-Phosdrin is indeed selective: the ratio LD_{50}(mouse)/LD_{50} (house fly) is about 25, as compared to about 2 for *trans*-Phosdrin.

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* Registered trademark Shell Chemical Corp., New York, N. Y.

The fact that dimethoate and acethion amide are selective strongly suggests that an amidase is more vigorous in the mammal than the insect, and therefore that in general the introduction of a carboxyamide group into a phosphorothionate should also confer selective toxicity.

TABLE V
Selective Toxicity to Insects Bestowed by Carboxy Ester Introduction (60)

	L_1 = LD_{50} mouse (intraperitoneal), mg./kg.	L_2 = LD_{50} house fly (topical), mg./kg.	L_1/L_2	pI_{50}[a] serum cholinesterase
Acethion $(C_2H_5O)_2P(S)SCH_2COOC_2H_5$	1280	9.4	136	5.4
Acetoxon $(C_2H_5O)_2P(O)SCH_2COOC_2H_5$	214	3.4	63	7.7
Prothion $(C_2H_5O)_2P(S)SCH_2CH_2COOC_2H_5$	2600	104	25	2.2
Propoxon $(C_2H_5O)_2P(O)SCH_2CH_2COOC_2H_5$	435	57	7.6	5.6
Methyl methylprothion $(C_2H_5O)_2P(S)SCH_2CH(CH_3)COOCH_3$	ca. 2000	60	ca. 33	4.5
Acethion amide $(C_2H_5O)_2P(S)SCH_2CONH_2$	200	4	50	2.5
Malathion (for comparison)	815	12	68	2.0

[a] Negative logarithm of molar concentration required to inhibit the enzyme 50% (therefore large for potent inhibitor).

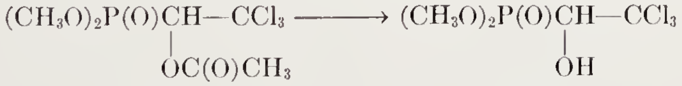
The fact that diazinon, Co-Ral, and ronnel are selectively toxic has some most interesting implications. If degradative differences are involved, this must mean that the appropriate phosphatases must be more active in mammals than insects since these compounds are degraded primarily by phosphatases. But the majority of the nonselective organophosphates (e.g., TEPP, parathion, and DFP) are also broken down by phosphatases, and therefore it is probable that these phosphatases are not substantially more active in mammals than in insects. There must therefore be two phosphatases or (far more likely) two classes of phosphatases, only one of which is more active in mammals than insects.

Now the common organophosphates nearly all contain either $(C_2H_5O)_2\overset{\|}{P}—$ or $(CH_3O)_2\overset{\|}{P}—$ groups, and we find both kinds of group present in selective and nonselective compounds. The same is true of the $\equiv P(O)$ and $\equiv P(S)$ groups, and the $=\overset{\|}{P}—O—$ and $=\overset{\|}{P}—S—$ groups. It follows that the special feature of the compounds which are degraded by the class of phosphatases which is more active in mammals must be in the side chain, X, of compounds, regardless of whether the type is $(RO)_2P(O)OX$, $(RO)_2P(S)OX$, $(RO)_2P(O)SX$, or $(RO)_2P(S)SX$.

We should therefore try to find the substrate specificity of this useful class of phosphatases; i.e., we should establish the kinds of group X which bestow the special property of being broken down by the phosphatases which are more active in the mammal. In such a search it is irrelevant at first to consider the toxicity, insecticidal action, or economics of the compounds studied. The first aim should be simply to be able to say "when X has the following properties, $(RO)_2P(S)OX$, etc., will be degraded by a phosphatase which is particularly active in mammals." The final step will be to find toxophoric groups to which the selectophore X may be attached.

The principle underlying the work just described might be formulated as follows: selective toxicity may be obtained by introducing into a toxophoric nucleus a selectophoric group whose removal constitutes a detoxification, and which can be more readily removed by the friend than by the enemy.

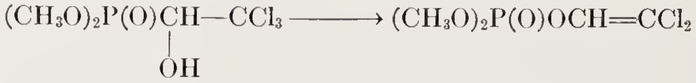
Another principle at once suggests itself: one could introduce into a potential toxophore a group whose removal constitutes activation of the toxophore, and which can be more readily removed by the enemy than the friend. An example is found in the case of acetyl-Dipterex (II), which inhibits acetylcholinesterase by 50% at $6.3 \times 10^{-4}M$ (3). Hydrolysis of this compound yields Dipterex (III) which



(II) (III)

$$(CH_3O)_2P(O)\underset{\displaystyle OC(O)CH_3}{\underset{|}{CH}}-CCl_3 \longrightarrow (CH_3O)_2P(O)\underset{\displaystyle OH}{\underset{|}{CH}}-CCl_3$$

is a more potent anti-cholinesterase than acetyl-Dipterex, causing 50% inhibition at $3.2 \times 10^{-4}M$. Just why this hydrolysis is an activation is uncertain. Arthur and Casida (3) believe that the free hydroxyl of Dipterex aids in hydrogen bonding to the cholinesterase. Metcalf *et al.* (45) believe that the anticholinesterase activity of Dipterex is due to dehydrochlorination and rearrangement to DDVP (IV). Whichever is true, acetylation of the hydroxyl would reduce



$$(CH_3O)_2P(O)\underset{\substack{|\\ OH}}{CH}{-}CCl_3 \longrightarrow (CH_3O)_2P(O)OCH{=}CCl_2$$

(III) (IV)

the anticholinesterase activity. This example also demonstrates a case in which hydrolysis constitutes activation rather than degradation.

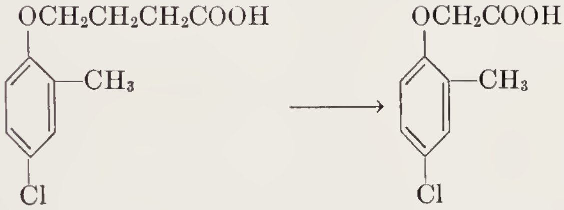
One would predict that if two species were equally susceptible to Dipterex, but one had more of the deacetylating enzyme, the species with more enzyme would be more susceptible to acetyl-Dipterex than the other (that is, the reverse of the malathion situation). Unfortunately, we do not have such a convenient pair of species. All we can say is that acetylation of Dipterex increases the LD_{50} (intraperitoneal) for rats from 400 to 800 mg./kg., but only increases the LD_{50} (topical) from the house fly from 11 to 12 mg./kg. (3). A reasonable guess would therefore be that the deacetylating hydrolysis occurs with greater ease in the house fly than in the rat.

Are these principles applicable to other pesticides? Certainly they are, once we can establish for *any* pesticide what sort of biochemically feasible reactions (such as hydrolysis, oxidation, reduction, decarboxylation) can constitute either an activation or a degradation. The next need is to find what useful variations exist in partially nonspecific reactions of this kind between friendly and inimical species. Part of the search must therefore be a rather routine affair: one takes the friend and the enemy and uses a battery of substrates on them both. When it is found that a particular substrate is modified strongly by one and not the other, one should examine analogues of this substrate to determine how far one could go with introducing the significant group as a selectophore into various toxophoric nuclei. Finally, one designs a molecule so that the enemy can activate the toxophore more readily than or degrade it less easily than the friend.

An important point is that this approach does not require knowledge of the mode of action of a compound, although such knowledge would make the problem easier. The existence of a large body of data on the effectiveness of numerous structural modifications would be particularly useful, since one would then know (for instance) what compounds could be arranged to be the inactive end product of metabolism in the friend. Such bodies of data undoubtedly exist for many pesticides, even though hidden in company files.

For the sake of completeness a very familiar example of selectivity conferred by degradation should be mentioned: DDT resistance is very probably attributable to the high levels of DDT-dehydrochlorinase in resistant insects (34,35) which lead to more extensive degradation of DDT in the resistant strains of house fly (65,72) and *Anopheles sundaicus* mosquito (34).

An excellent example in another sphere is the use made by Wain (92,93) of the opposite principle: the compound γ-(2-methyl-4-chlorophenoxy)-butyric acid (V) can be oxidized to the toxic acetic analogue (2-methyl-4-chlorophenoxy)-acetic acid (VI), by the nettle or thistle, which thereby kill themselves. Clover or celery cannot carry out this oxidation and are therefore not damaged by the compound.



(V) (VI)

C. DIFFERENCES IN DISPOSAL

1. *Excretion*

The mammal has an excretory system of an extremely advanced and efficient kind, and one might expect that it would excrete toxicants (as well as other compounds) more rapidly and completely than does the insect. However, Brodie *et al.* (9) state that "urinary excretion is relatively unimportant in limiting the action of drugs, most of which must undergo chemical change before they can be excreted in appreciable amounts . . . lipid-soluble drugs would remain in the body almost indefinitely if the organism did not have ways of letting them escape into the urine as less lipid-soluble derivatives." This may account for the observations, recorded above (Fig. 1), that in poisoning with the relatively nonselective compound parathion the total chloroform-extractables (mostly the very apolar parent compound) in the body are substantially similar in insects and mammals, whereas the more polar metabolite paraoxon rises rapidly to a peak in the mammal and then soon disappears, but is far more persistent in the insect. With the ionic degradation products of organophosphates the difference between insects and mammals is even more profound: the peak level of these products in mammals is often achieved in 30 minutes after injection, and is followed by a precipitous decline as urinary excretion occurs (38,39).

However, over longer time periods the difference between insects and mammals is less. Roan *et al.* (76) showed that after applying a large dose (about 15 μg./g.) of TEPP to the American cockroach, about 50% was excreted in 10 hours. A lower dose (2 μg./g.) of paraoxon was only 10% excreted in 10 hours, and 50% in 3 days. Probably the per cent excreted in a given time is very dependent upon dose, being larger with a large dose. For instance, the mouse excretes within 4 hours after treatment 62% of Dimethoate given at 0.5 μg./g. in contrast to 93% of Dimethoate given at 30 μg./g. (39).

The figures in the last paragraph could be very misleading if used as direct indices of excretory ability. What they reflect is a combination of degrading plus excreting capacity. And since it is true of both insects (23,76) and mammals (13,16,37) that only tiny amounts of unhydrolyzed organophosphates are excreted, excretion can be of very little importance in deciding toxicity (this does not apply to the small number of ionic anticholinesterases). The determination of total amount excreted in different species may, however, give a fair indication of whether differences in toxicity are attributable to differences in metabolism, since high excretion rates probably imply high degradation rates. The same argument applies to different compounds fed to one species. For example, when Phosdrin is fed to cows, the urinary output is almost completed in 1 day (15), whereas when Ronnel is fed to cows or goats, urinary output continues for about 1 week (70). This difference is undoubtedly a reflection of degradation rate differences and has nothing to do with differences in the "excretability" of the degradation products or the parent compounds.

Ionic anticholinesterases are relatively few in number; the principal examples are listed in Table VI. In these cases excretion is probably an important route of disposal. But because of the ion barrier

TABLE VI
Toxicity of Cationic Anticholinesterases[a] (55a,56)
(LD_{50} at 24 hrs., mg./kg.)

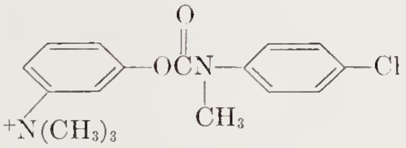
	Mouse[b]	House fly[c]	American cockroach[c]	Squash bug[c]	Milkweed bug[c]
Nu 1250 (VII)	0.3	590	890	300	>250
Nu 683 (VIII)	1.0	560	790	>500	>1000
Ro 30340 (IX)	8		>1000	600	800
Ro 30412 (X)	0.4	107	>1000	350	>500
Isosystox methosulfate (XI)	3	100	300	43	>500
Quaternarized Amiton* (XII)	0.17	>1000	400		

[a] Inactive anions not shown.
[b] Intraperitoneal.
[c] Intrabdominal.

of insect nerve (Section III.D.1) these compounds have no insecticidal activity. It is possible that to a class of animals which lack the ion barrier, yet have an excretory system less effective than the mammal, ionic anticholinesterases (or, indeed, any ionic poison) might show toxicity greater than to the mammal.

2. Storage

It appears that DDT and its metabolite DDE are extensively stored in the fat of mammals (28). In the American cockroach a



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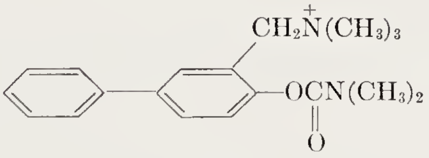

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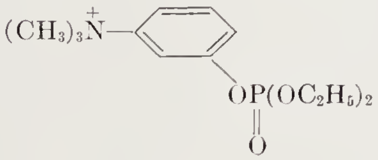

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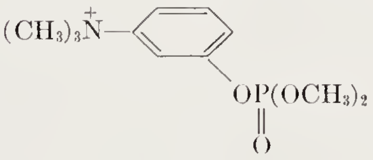
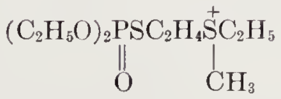
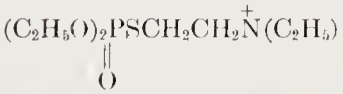


(X)



$(C_2H_5O)_2\underset{\underset{O}{\|}}{P}SC_2H_4\overset{+}{\underset{\underset{CH_3}{|}}{S}}C_2H_5$

(XI)



$(C_2H_5O)_2\underset{\underset{O}{\|}}{P}SCH_2CH_2\overset{+}{N}(C_2H_5)$

(XII)

* Registered trade name Imperial Chemical Industries Ltd.

relatively small fraction of the applied dose was recovered in the fat (77). However, storage appears to be more extensive in tolerant stages of American cockroaches (48). Since resistant house flies can tolerate body levels of DDT well in excess of the amount that would be fatal to susceptible house flies (65), it is probable that the resistant insect stores an important fraction of the applied dose. Storage may therefore play some role in determining resistance to DDT.

We have insufficient knowledge of the factors that influence excretion and storage, either in insects or in mammals. It is quite possible that a few simple parameters are of critical importance, and probably the partitioning properties are of major significance. For instance, parathion has an extraordinarily high lipid-water partition coefficient (for triacetin, about 6900; cf. malathion, 39 (55a)) which may account for the anomaly that it is toxic to mammals in spite of the presence in blood and liver of high levels of paraoxonase. It would be interesting to study a number of stable (i.e., not readily metabolized) compounds of graded polarity and find to what extent they are excreted or stored in insects or mammals. Such knowledge is a prerequisite for any reliable predictions as to the fate of a given compound in the body. We may guess, however, from the scattered data in the literature, that very polar compounds (such as salts) may be rapidly excreted and very apolar compounds (such as DDT) readily stored.

D. DIFFERENCES IN PENETRATION TO THE TARGET AREA

1. *Ionized Compounds*

In 1946 Tobias *et al.* (88) observed that acetylcholine was virtually nontoxic to the American cockroach, an observation that has now been extended to several other insects. Characteristic LD_{50}'s (in mg./kg.) by injection are: locust, >10,000; house fly, American cockroach, squash bug, and milkweed bug, all >2000 (30,56). These may be compared with corresponding mammalian figures of 150 (mouse, intraperitoneal), 20 (mouse, intravenous), 22 (rat, intravenous), 0.15 (rabbit, intravenous) (56,84). This sharp difference between insects and mammals suggested to Hopf (30) in 1952 that there was an intrinsic difference between the nervous systems of insects and mammals (for it is by a disruption of the nervous system that acetylcholine kills mammals). There are several objections to this radical view.

In the same year Hoyle (32) explained another anomaly, that the intact insect nerve is very insensitive to potassium concentration, yet the mammalian nerve is highly sensitive. Hoyle found evidence of a sheath around the peripheral nerve fiber of the locust which protected it from high potassium concentration. This sheath was absent in the mammal. It was not unreasonable to imagine that the potassium barrier would protect the nerve from other cations, of which acetylcholine (XIII) is an example.

$$(CH_3)_3\overset{+}{N}CH_2CH_2OC(O)CH_3$$

(XIII)

In 1956 it was shown by Twarog and Roeder (91) that the American cockroach nerve cord was invested with a sheath impermeable to sodium, potassium, and acetylcholine. When the sheath was stripped off, the ganglia were readily blocked by raising the concentration of potassium, lowering that of sodium, or by applying acetylcholine. Subsequently Yamasaki and Narahashi (97) have shown that when the sheath is broken, the response to ions of the American cockroach axon is precisely like that of the mammal.

In 1956, also, O'Brien (50,51) suggested independently that acetylcholine could not penetrate to cholinesterase (presumably located mainly in the nervous system), since acetylcholine was not hydrolyzed by the perfused roach, in spite of the considerable amount of cholinesterase shown to be present. In contrast, unionized substrates were readily hydrolyzed by such a preparation. It was pointed out that such an ion barrier could account for many anomalies in toxicity, such as the failure of the ionized compounds acetylcholine, *d*-tubocurarine, decamethonium, and prostigmine to be toxic to insects, in spite of their high toxicity to mammals, for which all these compounds are neurotoxic agents.

By now a great deal of evidence supports the idea that cationic compounds cannot penetrate to the intact ganglionic or peripheral nervous tissue of insects:

1. Twarog and Roeder (92) extended their observations on the ganglion of the American cockroach; such cationic compounds as urocanylcholine, decamethonium, tetramethylammonium, and benzoquinonium all blocked electrical activity in the desheathed ganglion

at high concentrations (e.g., $10^{-2}M$) which had no effect on the intact ganglion.

2. Harlow (27) showed that the cationic anticholinesterase prostigmine blocks the locust ganglion 100 times better if injected than if applied topically, yet the unionized compound TEPP is equally effective by either route.

3. Colhoun (15) showed that isolated eserinized American cockroach cords containing about 70 μg./g. of acetylcholine lost only a trace to their perfusing fluid; but if the ganglia were mutilated, 9 μg./g. of acetylcholine escaped.

4. Winton *et al.* (96) showed histochemically that acetylthiocholine cannot reach the cholinesterase of the intact American cockroach nerve cord, but can penetrate very locally where the nerve sheath is broken.

5. O'Brien and Fisher (56) showed that cationic anticholinesterases are far less toxic by injection to insects than to the mouse (Table VI).

6. O'Brien (55a) showed that TEPP, which is unionized, inhibits cholinesterase in intact nerve cords of the American cockroach as readily as in excised cords; but the quaternary analogue of Amiton (see Table VI) is a poor inhibitor with intact cords, although an excellent inhibitor with excised cords.

These six pieces of evidence, each based on a different approach present a remarkably consistent picture. We can say with confidence that it is generally true that cations penetrate very poorly to the insect nervous system.

What is the situation with respect to mammals? Since acetylcholine and the compounds of Table VI are so toxic to mammals, it is clear that ionization is no bar to toxicity. In general, however. ionic compounds do not penetrate to the mammalian central nervous system. It is therefore almost certain that the above compounds kill mammals by a peripheral action, probably by blocking neuromuscular transmission and causing asphyxia due to failure of the respiratory apparatus.

The insect neuromuscular junction appears to be insensitive to organophosphates (27). This, then, is the physiological difference which confers selectivity on the cationic anticholinesterases: their cationic nature forbids them entrance to the mammalian central nervous system or to the analogous insect ganglion, and the difference in neuromuscular sensitivity becomes of paramount importance.

It would be interesting to find whether anions have difficulty in penetrating the insect nervous system. One would guess that they would. The question does not arise in the case of anticholinesterases because anionic compounds are poor anticholinesterases due to the weakened electrophilic character of the phosphorus. However, if the anion were far away from the phosphorus, and kept far away by a rigid nontransmitting system, e.g., of heterocyclic and unconjugated rings, one might obtain an anionic anticholinesterase, and could examine its selectivity.

This section may be concluded with the following generalization: among anticholinesterases, and probably among all neurotoxic compounds, the presence of a cation induces strong selectivity such that mammals are poisoned but insects escape. Such compounds could be called *selective mammalicides.*

2. Ionizable Compounds

The above discussion has dealt with compounds which are salts and are ionized in any aqueous system regardless of pH. A very important group of compounds (including for instance the huge family of biologically active amines) has the property of varying in extent of ionization with pH; these are the acids and bases, and it is the weak bases which we need to consider here.

The following brief discussion about pK should be considered only as a useful (I hope) indication of the behavior of bases, rather than as a precise formulation of the actual mechanism. It will lead to the same approximate numerical conclusion as the precise treatment.

A base can be defined as a compound which can accept a proton (which is effectively the same as a hydrogen ion). Therefore in an aqueous system all bases can react thus:

$$\underset{\text{(free)}}{B} + H^+ \rightleftharpoons \underset{\text{(protonated)}}{BH^+}$$

At any time some of B is free, some is protonated as BH^+. The amount protonated depends upon the pH of the system and on the base strength of B. Strong bases are protonated even at high pH; they can seize protons even when these are short supply. Weak bases are protonated only at low pH. The base strength is expressed by the pK_a, which is equal to the pH at which 50% of the base is protonated; thus strong bases have high pK_a's. At 1 pH unit below its pK_a, about 90% of any base is protonated; at 1 unit above the pK_a about 10% is protonated.

It is reasonable to expect from what was said in the previous section that the protonated form, BH^+, will not penetrate to the nerve cord, but the free base form, B, will. If this is true, there should be two consequences: (*a*) one pH (i.e., physiological pH), various bases: neurotoxic agents (such as anticholinesterases) which are basic will be selectively toxic to mammals; the selectivity will be extreme with strong bases, because these will be mainly protonated, and less with weak bases; (*b*) one base, various pH's: the capacity of any one base to penetrate the insect nerve cord should increase with pH, since less is protonated at high pH. There is evidence that both these consequences are indeed found.

(*a*) *Various Bases at Physiological pH.* We would like to show that, within a series of bases, as pK_a increases penetration of the insect nerve cord decreases. Unfortunately, the intrinsic activity of the compounds will probably vary also, so this must be corrected for. A partially successful attempt was made by O'Brien and Fisher (56) by using toxicity to the mouse as the measure of intrinsic effectiveness, since in the mouse ionization should not affect penetration (i.e., to peripheral nervous systems). If the *only* difference between the effect of an agent in the insect and that in the mouse were due to penetration, it was suggested as a first approximation that the ratio effectiveness(insect)/effectiveness(mouse) might be equal to the fraction unionized. Thus, if both insect and mouse have a body pH of 7, consider a base of $pK_a = 7$. One-half of the base would be ionized in the body; therefore the compound should be half as active in the insect as in the mouse, and a toxicity ratio, LD_{50}(insect)/LD_{50} (mouse), of 2 is predicted.

A study was made of 35 compounds which were neurotoxic to mammals, and their toxicities to the mouse were compared with those for five insect species. Table VII shows that in several cases quite good agreement between prediction and observation was found, i.e., within one order of magnitude. However, in very many cases the results threw little light on the phenomenon (e.g., a predicted ratio of 10^5 can, of course, never be achieved in practice as this would demand an insect LD_{50} of perhaps 100,000 mg./kg. or more, and at much lower doses one gets death due to unspecific effects such as hypertonicity); in several cases prediction and observation were radically different. In at least two groups, the convulsants and the narcotics, this failure was probably due to a fundamental physiological difference between insect and mammal.

TABLE VII

Agreement of Prediction and Observation for Selective Toxicity of Ionizable Neurotoxic Compounds[a] (56)

(LD_{50}(insect)/LD_{50}(mouse) at 48 hrs.)

		Observed			
Compound	Predicted	House fly	Cockroach	Squash bug	Milkweed bug
Nicotine	14	25	28	25	7.3
Tolserol	1	2.5	1.7	1.3	0.1
Pilocarpine	2.6	4	9	0.07	0.1
Diparcol	2.3	2.5	2.6	4	4.8
Noradrenaline	112	50	40	50	50
Lobeline	11	37	50	10	28
Nu 1250 (formula VII)	10^5	1700	1700	730	670

[a] All figures are by injection. Data for pea aphid omitted since LD_{50}'s for aphids were by contact.

The validity of the predictions is also open to question on the following grounds: if the ionizable compound was in the hemolymph and separated from the nervous system by an ion-impermeable barrier, the unionized molecules would cross the barrier; the remainder in the hemolymph would re-establish equilibrium, providing more unionized molecules and so on, so that in spite of the barrier there would *eventually* be the same concentration inside and outside the nerve cord. Thus, if all that mattered was the eventual concentration (and if there were no degrading enzymes), all ionizable compounds should show a toxicity ratio of 1 if their mode of action was the same in insects and mammals. However, the pK_a would affect the time involved in reaching equilibrium: thus in two bases whose pK_a's were 8 and 9 the initial rate of penetration into the cord would be ten times greater in the compound of pK_a 8, since 10% of it would be unionized at pH 7, compared to 1% of the compound of pK_a 9. Now, if degrading enzymes were important, and were similar in insects and mammals, the original prediction would be more accurate, for it would be the amount reaching the target in the early stages which would dictate toxicity, and this amount would depend upon the rate of penetration. The argument outlined above therefore represents an extreme condition, where the rate of penetration is all important; for the other extreme, where rate is entirely unimportant, the toxicity ratio should be 1. In practice, conditions probably fall somewhere between the two extremes. In view of all these considerations, it is perhaps surprising that any compounds obeyed the prediction!

The matter can only be cleared up by direct experiments: several biologically stable, radioactive bases of varying pK_a should be used, and the rate of appearance in the nerve cord after injection studied.

(*b*) *One Base at Varying pH.* As mentioned above, TEPP penetrates the intact nerve cord of the American cockroach with ease, quaternary Amiton with great difficulty. In neither case was the penetration affected by pH (between pH 5 and 9). Tetram* is the oxalate salt of Amiton, and the active part is:

$$(C_2H_5O)_2P(O)SCH_2CH_2N(C_2H_5)_2$$

It is a weak base with a pK_a of 8.4, and is therefore 50% ionized at 8.4, and more ionized at lower pH. One would predict that its excellence in penetrating the nerve cord would vary with the pH in the range 7.4–9.4, being best at high pH. Experiments (55) showed that this was qualitatively true. Figure 3 shows the results: the internal level was calculated from the inhibition of cholinesterase in the cord, following an incubation of the intact cord with Tetram. The figure shows also the variation of the unionized fraction with pH (calculated from the pK_a). It is apparent that the internal level varies qualitatively as the unionized fraction, but that the correspondence is not complete.

The cause of the lack of overlap of the curves in Figure 3 is probably found in the factors discussed in Section III.D.2(a). As the unionized molecules enter the cord, the external Tetram "generates" more of the unionized form, so that eventually the internal level should equal the external level; but the rate of achievement of this equality will vary with pH, and this variation should follow the "per cent unionized" curve of Figure 3. Now, the cholinsterase inhibition technique does not measure the increment of penetration within a small time increment; if it did, the curves of Figure 3 should overlap. Instead, it detects what penetrates during 1 hour, yet gives extra

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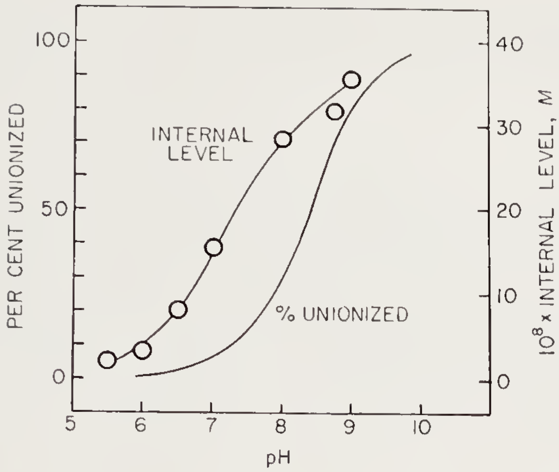


Fig. 3. Influence of pH upon ionization of Tetram and upon penetration of Tetram into the intact nerve cord of the American cockroach. Internal level of Tetram calculated from cholinesterase inhibition following 1 hour exposure of intact cord. (Data of O'Brien, 55a)

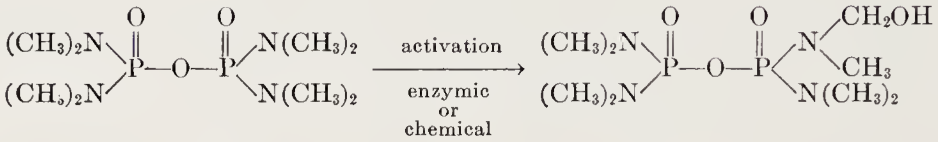
"consideration" to the molecules that arrived first, since they have an effective incubation time with cholinesterase greater than the latecomers. Consequently, ionization is not quite so unfavorable as it would be in a true rate-measuring technique, and this perhaps explains why the experimental curve is to the left of the "per cent unionized" curve. One obtains a similar effect, and for the same reason, by extracting Tetram with chloroform from aqueous solutions of various pH. For a fixed small extraction time (which has to be rigidly controlled) one obtains a variation with pH quite like the "internal level" curve of Figure 3.

We can say, however, that pH profoundly affects penetration of this base into the nerve cord (other experiments showed that it was indeed penetration and not inhibition itself that was affected by the pH) and that a base penetrates best at high pH. This knowledge confirms the earlier conclusion that cations penetrate poorly. It also makes possible the following generalization: in neurotoxic compounds which do not affect the insect neuromuscular junction the presence of a basic group confers selective toxicity toward mammals as compared with insects; the higher the pK_a of the base, the more selective is the compound likely to be.

It is not anticipated that a fervent need for such selective mammalicides will arise! However, the generalization can be immediately useful in suggesting which compounds the insecticide designer should avoid.

3. *Unionized Compounds*

The selectivity of schradan (XIV) has long been a mystery: it is intrinsically toxic to plant-sucking insects such as aphids, mites, and the true bugs (e.g., squash bug, milkweed bug) and also to mammals, but is virtually nontoxic to flies, cockroaches, beetles, caterpillars, and most other insects (57,68). It is now considered that the toxic principle is the hydroxymethyl metabolite (hydroxymethylschradan, XV) (29,86) and not the *N*-oxide as had been previously believed (14).



(XIV) (XV)

There have been various theories as to the reasons for the selectivity of schradan, none of which (including our own) have been particularly convincing (14,57). The possibilities that activation is particularly vigorous in susceptible insects, or that degradation is particularly vigorous in nonsusceptible insects, have been excluded (57,58). O'Brien and Spencer (59) have now shown that the American cockroach (for which the LD_{50} of schradan is 1340 mg./kg.) can produce ample hydroxymethylschradan to inhibit its cholinesterase completely, but that the metabolite penetrates the nerve cord so poorly that the cholinesterase is in fact little inhibited. If one mutilates the nerve cord, the metabolite penetrates fairly readily. It is therefore probable that the failure of schradan to poison most insects is due to the failure of its active metabolite to penetrate the nerve cord.

But why are the susceptible insects killed? Possibly they have a less protected nervous system, but there is no evidence on this point.* It may be significant that the base Amiton has a pattern of toxicity very like that of schradan: both kill mites, aphids, and mosquito larvae but are not toxic to cockroaches, houseflies, and probably flour beetle and lady beetle (8,49). These data suggest that these

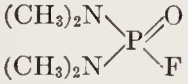
* Saito (79,80) has recently provided electron microscope and radioactive tracer data that confirm this hypothesis.

insects are ill-protected against schradan or basic compounds. Yet the schradan-susceptible milkweed bug and squash bug are relatively unaffected by cationic anticholinesterases (56), so their nerve barrier has at least some protective value.

There is another piece of evidence in favor of the view that the selectivity of schradan is due to a penetrative difference. In the two previous sections it has been shown that the whole of the organophosphate-sensitive part of the insect nervous system is protected by a barrier which behaves just like the "blood-brain" barrier of the mammal. Now, when a mammal is poisoned by schradan (or by its analogues which contain dimethylamido groups), there are no signs of disturbances of the central nervous system, nor is the brain cholinesterase inhibited (5,21,22,61). Therefore the hydroxymethylschradan cannot penetrate the blood-brain barrier of the mammal; it is then reasonable that it should not penetrate to the nervous system of the insect.

The work with schradan analogues suggests that a single dimethylamido group in such a molecule confers the impermeability with respect to the mammalian central nervous system. It should then confer selective toxicity toward schradan-susceptible insects and mammals (once again this may not be a very exciting selectivity from the economic viewpoint!). It would be interesting to know if other amidic groups also confer this selectivity.

The extremely limited data available suggests that dimefox (XVI) and mipafox (XVII) do not show this selectivity (31,49). However,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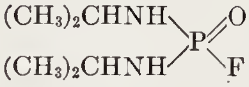
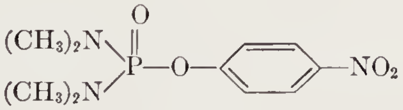

(XVII)

Metcalf and March (46) have shown that *O-p*-nitrophenyl *N,N*-dimethyl phosphorodiamidate (XVIII) has a toxicity pattern similar


(XVIII)

to schradan in that it is nontoxic to the bee, house fly and cockroach, the LD_{50} being, respectively, >1000, >500, and >100 mg./kg. (46), but is toxic to the mouse, with an oral LD_{50} of 7 mg./kg. (49).

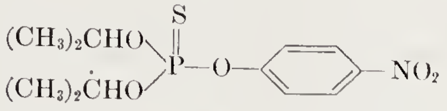
Clearly, this whole topic requires investigation. What are the physical or biochemical properties which are responsible for the failure of the activation products of schradan and its analogues to penetrate insect nerve or the mammalian central nervous system? Why do phosphoramide groups have one effect in phosphoric anhydrides and aromatic phosphates, another in phosphorofluoridates? The answers to these questions will enable us to add one more to our list of principles of selectivity. We should be able to say: the presence of an alkyl phosphoramide group in a certain class of organophosphates confers selective toxicity toward mammals, Hemiptera, Homoptera, and mosquito larvae, as compared with other insects.

E. DIFFERENCES IN ATTACK ON THE TARGET

Some of the factors involved when a poison attacks its target are: affinity of poison for the critical site; ease of attachment once orientated on the site; reaction when attached (including reactions with the target and those not immediately with the target but which modify the attachment); ease of spontaneous or induced removal from the site. For any one compound these factors may be influenced by the precise nature of the target (small steric or electronic differences could make profound differences in all of the above factors) and by its immediate environment, including such conditions as pH, temperature, ionic concentration, and the presence of reactivating or deactivating factors. If one of these factors is different in friend and enemy, there is the possibility of turning the difference to our advantage by designing an appropriate compound. Design along these lines demands knowledge of the nature of the target. This is known (among pesticides) only for the anticholinesterases and for certain fluorine compounds, about which nothing constructive can yet be said with respect to selective toxicity.

For the purposes of this section it will be assumed that the target of organophosphates in insects, as well as in mammals, is cholinesterase. The evidence for this assumption was reviewed in 1957 (85). Two pieces of evidence have since been published which throw some doubt on the matter (4,31), but these cannot be evaluated in the space available here.

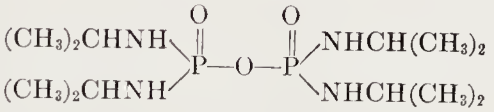
The main question resolves itself to: are there differences in cholinesterase between species which can confer selectivity? There probably are. Metcalf and March (46) found in 1949 that diisopropyl



(XIX)

p-nitrophenyl phosphorothionate (XIX) was over 250 times as toxic to the housefly as to the bee, and was about 1000 times more potent against the cholinesterase of fly brain than that of bee brain *in vitro.* This important observation needs confirmation for two reasons: it is now known that phosphorothionates require oxidation to become *in vitro* anticholinesterases, and it is probable that the *in vitro* activity observed was due to impurities. Also Metcalf *et al.* (47) showed in 1956 that the bee contains a large amount of an aromatic esterase which could hydrolyze parathion, the ethyl analogue of the above phosphorothionate, with great rapidity. It is therefore possible that the bee's immunity is due to a high degradative capacity. It is not *probable,* because parathion is very toxic to the bee, with an LD_{50} of 3.5 mg./kg. (47).

Studies have been made on the variations of susceptibility of various mammalian cholinesterases to organophosphates. Table VIII gives some examples. Although there are extreme variations in the susceptibility of true and pseudo cholinesterase, for any one enzyme the variation is not too extreme; e.g., for iso-OMPA (XX)



(XX)

and true cholinesterase there is a maximum variation between species of 1.26 units or 18-fold. One would presume that this variation would be reflected in a toxicity differential, but no information is available on this point.

It has been suggested by Casida *et al.* that the selectivity of schradan is due to the relative sensitivity of the cholinesterase of susceptible species to the active metabolite (12). However, there was only a 2-fold difference in sensitivity between the cholinesterase of the rat and house fly, although the LD_{50} of schradan for the rat is 8 mg./kg., for the house fly, >500 mg./kg. (21,46). The maximum difference in sensitivity of cholinesterases between susceptible and

TABLE VIII

Selectivity of Organophosphates to Cholinesterases of Various Mammals (2)

	Iso-OMPA (XX)			Isopestox (I)		
	pI_{50}[a]			pI_{50}[a]		
	True chE	Pseudo chE	I_{50}(true)/ I_{50}(pseudo)	True chE	Pseudo chE	I_{50}[a] (true)/ I_{50}(pseudo)
Horse	2.47	6.52	11,300	3.80	7.42	4,200
Human	3.52	5.27	56	4.66	6.41	56
Guinea pig	2.34	5.89	3,600	4.21	6.51	200
Dog	2.42	6.54	13,200	4.06	7.29	1,700
Rat	3.60	6.32	530	4.22	6.75	340
Sheep	3.20			4.41		

[a] I_{50} = molar concentration required to inhibit cholinesterase 50%; pI_{50} = negative logarithm of I_{50}.

nonsusceptible species was 50-fold, or 1.7 pI units; this is a rather small difference.

Van Asperen and Dekhuijzen (4a) found a vital difference between house fly and mammalian cholinesterase: the insect enzyme fails to recover spontaneously from inhibition *in vitro* by DDVP, whereas the mammalian enzyme recovers fairly rapidly. This phenomenon has been confirmed by Mengle and O'Brien (42) and shown to occur also with DFP, TEPP, parathion, paraoxon, malathion, and malaoxon. Yet Mengle and Casida (41) have shown *in vivo* that the inhibited enzyme of the house fly recovers rapidly if the animal survives, in fact the recovery is somewhat more rapid than in the mammal. Since it is *in vivo* conditions that are significant in poisoning, we cannot yet exploit these observations. But a fundamental difference in the nature of the cholinesterases is implied. If we can locate that difference precisely, it should be possible to utilize it to design selective poisons.

IV. The Future

So far, the design of selective insecticidal compounds has only been possible with organophosphates. There is no reason why some of the principles discussed above should not be extended to other toxophores. The chlorinated hydrocarbons are one possible group; but studies on their mode of action have been so unyielding as to offer little hope in the near future for confident predictions. Probably it is mammal-insect selectivity that is easiest to tackle, and most of the chlorinated hydrocarbons do not have much mammalian hazard.

The carbamates will undoubtedly be extensively studied in the next few years, and since their target is probably cholinesterase, there is every hope that their selectivity can be put on a rational basis.

We need new toxophores. Any compound which is toxic to mammals presents a good starting point: once one has the essential toxicity, it should be possible to "build in" the selectivity. Perhaps the most promising candidate group is the fluoro acids and their derivatives: the years of brilliant study by Peters and his collaborators have given us an insight into the precise target of these compounds and the way they are metabolized by mammals (67). There is a recent report by David (17) that a degree of selectivity (i.e., aphicidal activity with reduced mammalian hazard) is bestowed upon sodium fluoroacetate (XXI) simply by forming its amide (fluoroacetamide, XXII). Here is the most promising of beginnings for a whole new series of studies upon selectivity.

FCH_2COONa
(XXI)
(Nonselective)

FCH_2CONH_2
(XXII)
(Selective)

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POTENTIATION OF THE TOXICITY OF ORGANOPHOSPHORUS COMPOUNDS

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I. Introduction

The continued development of new organophosphorus insecticides is an indication of the value of this group of toxicants for the eradication of destructive insects. Although there are some toxicological problems associated with the use of these agents, they must be viewed from the standpoint of achieving a balance between the beneficial effects of the compounds to agriculture and the health hazards in the manufacture and application of these insecticides as well as in the consumption of foods upon which they have been used. To accomplish this objective knowledge is required of the toxicity of organophosphorus compounds to mammals under conditions comparable to those under which exposure occurs during their practical use.

The most important organophosphorus insecticides, particularly those for which tolerance levels have been established, have been studied extensively in experimental animals with respect to their acute, subacute, and chronic effects. It seems likely that few, if any, significant pharmacological effects of these compounds on animals have escaped detection. The permissible levels (tolerance levels) of the organophosphates in foods were carefully established on the basis of the known degree of toxicity of each compound. When the levels were established, consideration was given to the probability that combinations of these insecticides would have strictly additive toxic effects, since they have a similar mechanism of action, but an additional margin of safety was included for the unknown and unexpected circumstance which might accentuate toxicity.

Until recently there was no evidence that more than strictly additive toxic effects result from combinations of organophosphorus insecticides. However, in 1957 Frawley *et al.* (5,6) undertook experiments to ascertain whether potentiation of toxicity results from the simultaneous administration of combinations of organophosphorus insecticides to animals. These experiments were based upon the fact that people may ingest several food products, each containing a different organophosphate, at the same meal, and workers may be exposed to two or more compounds on the same or successive days during the manufacture or use of these materials.

At the present time several organophosphorus insecticides have been approved for use on food crops, the most important of which are listed in Table I together with the maximum levels (tolerance levels) that are permitted on food crops. Frawley and associates (5,6) selected two of these compounds, namely EPN (ethyl *p*-nitrophenyl benzenethionophosphonate) and malathion, for their investigation. The acute toxicity of the two compounds given simultaneously was determined using mortality as the end point. Potentiation of toxicity, when the compounds were fed in the diet to dogs and rats, was detected by performing periodic blood cholinesterase measurements. In the initial studies of Frawley and associates (5,6) the combination of EPN and malathion was administered in an approximate equitoxic ratio of 25 parts of malathion to 1 part of EPN. The LD_{50} of malathion given orally in single doses to rats was 1400 mg./kg., and the value for EPN was 65 mg./kg.; but when the compounds were administered together, the LD_{50} was 167 mg./kg. for malathion and

TABLE I

Tolerance Levels of Some Organophosphorus Insecticides

Organophosphate	Tolerance level, p.p.m.
Parathion	1
Methyl parathion	1
Systox*	0.75
EPN	3
Malathion	8
Guthion*	2
Phosdrin	1
Diazinon**	0.75
Trithion***	0.8
Octamethylpyrophosphoramide	0.75

6.6 mg./kg. for EPN, thus representing a 10-fold increase in the toxicity of each agent. If the two agents had exerted strictly additive toxicity, the LD_{50} of this particular mixture should have been 700 mg./kg. of malathion plus 32.5 mg./kg. of EPN (one-half of the LD_{50} of each compound). Similar but less extensive acute toxicity tests on dogs suggested an order of potentiation of approximately 50-fold. Following these observations subacute toxicity studies were undertaken in which rats were fed various levels of EPN plus malathion in the diet and blood cholinesterase measurements were made at intervals over a period of 8 weeks. The results of these measurements indicated that dietary levels of 25 p.p.m. of EPN and 50 p.p.m. of malathion caused an amount of inhibition of cholinesterase which was much greater than would be expected on the basis of strictly additive effects. Similar studies on dogs indicated that even the tolerance levels of each agent on fruits and vegetables for human consumption (3 p.p.m. of EPN and 8 p.p.m. of malathion) resulted in potentiated toxicity which was detectable by erythrocyte cholinesterase measurements. The effect at these low dietary levels was of borderline significance. Nevertheless, it constituted the evidence upon which to base the suggestion that potentiation of toxicity might result from the dietary intake of permissible levels of organophosphorus compounds in food. These experiments formed the basis for the recommendation by the

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* Registered trademark Chemagro Corporation, Kansas City, Mo.

** Registered trademark Geigy Chemical Corporation, Ardsley, N. Y.

*** Registered trademark Stauffer Chemical Co., New York, N. Y.

Food and Drug Administration that new organophosphates for which tolerance levels are desired should be tested acutely and subacutely for potentiation in combination with each of the other organophosphates for which tolerance levels have been established and with all other new compounds which are at a similar stage of development. However, as indicated in Section IV, studies on a number of pairs of organophosphates have indicated that agents which do not cause potentiation of toxicity acutely will likewise not produce the effect at the lower doses employed for subacute tests. For this reason acute toxicity tests are now employed to ascertain whether a pair of compounds is capable of producing potentiation. Those compounds which exhibit no potentiation acutely are given no further consideration. Subacute tests are performed only on those pairs of compounds which exhibit definite potentiation acutely.

II. Methods of Detecting Potentiation of Toxicity of Organophosphorus Insecticides

The detection of potentiation by acute toxicity tests may be accomplished by comparing the observed LD_{50} values, which are determined experimentally, with the expected values for various pairs of organophosphates. The simplest method of detecting potentiation acutely involves the simultaneous administration of equivalent fractions of the LD_{50} of each compound. If the administration of one-half of the LD_{50} of each of two compounds results in additive (50%) or less than additive mortality, it is apparent that no potentiation has occurred. In cases where potentiation does occur an indication of the magnitude of the effect can be obtained by further toxicity tests.

There are several methods which can be used for measuring the degree of potentiation. One of these methods involves determination of the LD_{50} of equitoxic mixtures. This is done by mixing the two compounds using concentrations of each which are related to their comparative toxicities. Thus, if the LD_{50} of one component of the mixture is 10 mg./kg. and the LD_{50} of the other component is 90 mg./kg., a mixture containing 10% of one and 90% of the other compound, respectively, would be employed for the toxicity tests. Comparison of the observed LD_{50} with the value which would be expected on the basis of strictly additive effects provides an indication of the degree of potentiation. Another method of measuring the degree of potentiation by pairs of organophosphates involves the determination of the LD_{50} of various percentage mixtures containing two components. In this case the expected LD_{50} can be calculated by the method of Finney (4) using equation 1.

$$\% \text{ component } A/(LD_{50} \text{ of } A \times 100) + \% \text{ component } B/(LD_{50} \text{ of } B \times 100) = 1/LD_{50} \text{ of mixture} \quad (1)$$

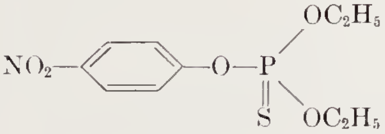
This method of calculation requires that the mechanism of action of the two compounds be similar and that the dosage-mortality curves be parallel. The organophosphates which have been studied thus far satisfy these requirements in that inhibition of cholinesterase appears to be the principal mechanism of action and the LD_{50} regression lines for cholinergic organophosphates are reasonably parallel. Expression of the degree of potentiation as the ratio of the observed to the expected LD_{50} values has the advantage of simplicity and provides an estimation of the degree of hazard. However, it should be emphasized that these procedures have been designed for application to situations in which the mechanism of potentiation is unknown. None of the procedures in use at the present time for calculating and expressing the degree of potentiation make any assumptions concerning the mechanism of potentiation and in fact their use constitutes a method of estimating the degree of hazard in the absence of specific information on the mechanisms involved. When information on the exact mechanism responsible for potentiation among various pairs of organophosphates becomes available, a much more precise estimation of the degree of hazard can be made. For example, if it is known that Compound A blocks the detoxification of Compound B, then the degree to which the toxicity of Compound B is magnified by the potentiating agent can be determined and expressed in a straightforward and readily understandable manner. This procedure requires knowledge of the mechanism of potentiation in each case, but until our knowledge advances to that stage it is necessary to utilize some generally applicable but less precise method of measuring the effects, such as those procedures which are in use at the present time.

The procedure suggested by the Division of Pharmacology of the Food and Drug Administration for the detection of potentiation of the toxicity of organophosphorus insecticides by subacute feeding experiments consists of the use of blood cholinesterase measurements to detect greater than additive toxicity. Pairs of organophosphates are fed at the maximum safe levels to one male and one female dog for 6 weeks. The safe level is defined as the maximum dietary level which will produce no inhibition of blood cholinesterase. Five normal plasma and erythrocyte cholinesterase measurements are performed within a 2 week period on each dog prior to feeding the experimental diets. Cholinesterase measurements are made at weekly intervals for 6 weeks during the period that the organophosphates are fed. By comparing the effects of each compound given alone with those produced by combinations of compounds, it can be determined whether potentiation occurs. Under these experimental conditions an amount of inhibition exceeding 40% is indicative of potentiation. Smaller amounts of inhibition can occur from simple addition of the toxic effects of the two compounds. A number of studies have been conducted using this procedure, and there has been no significant disagreement about the usefulness and feasibility of this method of testing for potentiation. However, as indicated in Section I, a sufficient amount of evidence has accumulated to indicate that it is unnecessary that subacute tests for potentiation be done on all organophosphates. This fact becomes evident later in this communication where it is indicated that potentiation of toxicity is due to inhibition by one agent of the detoxification of another compound. Under these conditions it is apparent that agents which do not inhibit detoxification enzymes at the high dose levels used in acute toxicity tests will not inhibit the enzymes when much lower levels are fed in the diet.

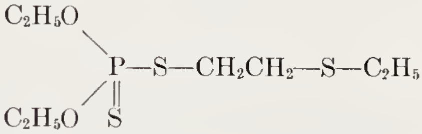
III. Use of Acute Toxicity Tests for Detection of Potentiation by Organophosphorus Compounds

Experiments have been conducted on a number of pairs of organophosphates to ascertain whether potentiation of toxicity occurs. These tests have revealed that three types of responses may be obtained. Thus, strictly additive, less than additive, and greater than additive effects (potentiation) can occur. The experience in this and other laboratories has been that most pairs of organophosphates produce either additive or less than additive acute toxic effects when mortality is used as the end point. With respect to the acute toxicity tests it is important to recognize that the dose of each compound is necessarily limited by its toxicity. Thus a compound which might be capable of inhibiting the detoxification of another organophosphate at high dose levels cannot be administered in sufficient quantity to exert this effect because its own toxicity becomes the dose-limiting factor. For this reason, and from the standpoint of assessing the practical hazard, the use of intact animals is superior to *in vitro* systems for testing for potentiation, since with the latter method evidence of potentiation can sometimes be obtained at dosage levels which could never be achieved in mammalian organisms.

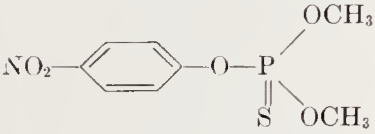
All of the organophosphorus insecticides for which tolerance levels have been established have been subjected to acute potentiation tests using rats as the test animal. In addition, a number of other compounds which are in various stages of development have been tested for potentiation by administration in combination with each of the other compounds that are in a similar or more advanced stage of development. Some of the compounds which have been subjected to acute potentiation tests, in this laboratory or by other investigators together with common and chemical names, are shown below.



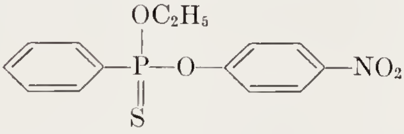
Parathion
O,O-Diethyl *O*-*p*-nitrophenyl
phosphorothioate



Di-Syston*
O,O-Diethyl *S*-[2-(ethylthio)ethyl]
phosphorodithioate

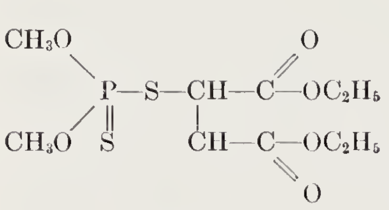


Methyl parathion
O,O-Dimethyl *O*-*p*-nitrophenyl
phosphorothioate

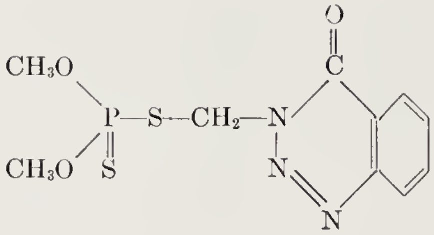


EPN
O-Ethyl *O*-*p*-nitrophenyl
benzenethionophosphonate

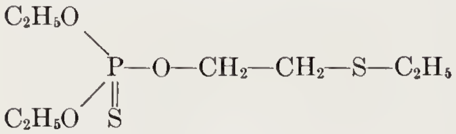
* Registered trademark Chemagro Corporation, Kansas City, Mo.



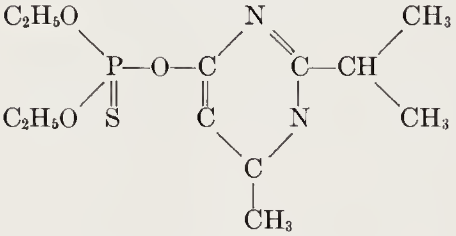
Malathion
S-[1,2-Bis-(ethoxycarbonyl)ethyl]
O,O-dimethyl phosphorodithioate



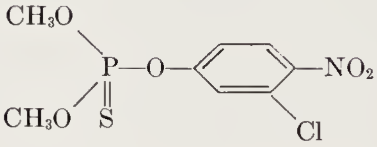
Guthion
O,O-Dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-yl)-methyl phosphorodithioate



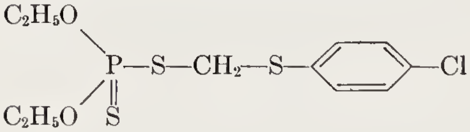
Systox
O,O-Diethyl *O*-[2-(ethylthio)ethyl]
phosphorothioate



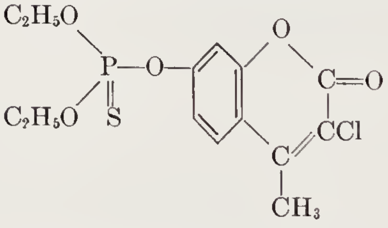
Diazinon
O,O-Diethyl *O*-(2-isopropyl-4-methyl-pyrimidyl-6) phosphorothioate



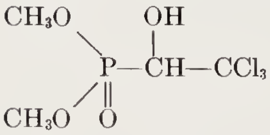
Chlorthion
O,O-Dimethyl *O*-(*m*-chloro-*p*-nitrophenyl) phosphorothioate



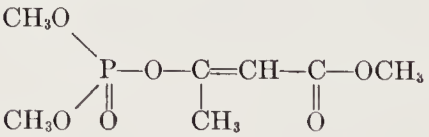
Trithion
S-(*p*-Chlorophenylthio)-methyl
O,O-diethyl phosphorodithioate



Co-Ral
O,O-Diethyl *O*-(3-chloro-4-methyl-coumarinyl-7) phosphorothioate



Dipterex
O,O-Dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate



Phosdrin
O,O-Dimethyl *O*-[(1-methyl-2-methoxycarbonyl)vinyl] phosphate (α-2-carbomethoxy - 1 - methylvinyl dimethyl phosphate)

In our experiments the large number of compounds studied made it desirable to utilize for the initial experiments the simplest method which would reliably detect potentiation. The procedure which was selected consisted of the administration of one-half of the LD_{50} of each compound in combination with the same fraction of the LD_{50} of another compound to groups of 20 rats. This method constitutes a reliable screening procedure by which the majority of pairs of organophosphorus compounds can be eliminated from further consideration with respect to potentiation. The pairs of compounds which exhibit potentiation by this test are studied in greater detail by carefully determining the LD_{50} of equitoxic mixtures.

Before undertaking potentiation tests it is necessary to measure the LD_{50} of each compound, and, since variations are often noted in the toxicity of different technical samples of a compound, the toxicity of the particular sample used for potentiation tests should be measured. The same solvent system and route of administration should be used for determining the LD_{50} and for the potentiation tests. The importance of accurate toxicity measurements on each sample prior to potentiation tests cannot be overemphasized since the results of the potentiation tests are dependent upon the value selected as the LD_{50} for each compound. For the tests conducted

TABLE II
Acute Toxicity of Several Organophosphorus Insecticides to Female Rats[a]

Organophosphate	Approx. LD_{50}, mg./kg.
Co-Ral	110.0
Dipterex	160.0
Systox	2.5
Di-Syston	2.0
EPN	7.2
Parathion	2.0
Methyl parathion	2.8
Guthion	5.7
Trithion	6.0
Diazinon	90.0
Phosdrin	0.8
Tetram	0.6
Malathion	800.0

[a] All compounds were given intraperitoneally.

in this laboratory the compounds were dissolved in a mixture of 20% ethanol and 80% propylene glycol, with the exception of malathion which was given in the undiluted form and Co-Ral* which was given as an aqueous suspension in 0.2% carboxymethylcellulose. The LD_{50} values for compounds used for potentiation tests in this laboratory are shown in Table II.

A. ADDITIVE ACUTE TOXICITY BY COMBINATIONS OF ORGANOPHOSPHORUS INSECTICIDES

When two compounds having the same mode of action, parallel dosage-mortality responses, and a similar time of onset of toxic effects are administered simultaneously at dosage levels equal to one-half their LD_{50}'s mortality in the neighborhood of 50% is the expected response. It is noteworthy, however, that the requirements for strict additivity are rather rigid in contrast to the apparent assumption by many investigators that strict additivity is an expected response from two compounds having the same mode of action. The data presented in Table III give the results of potentiation tests conducted in this laboratory, which showed that strictly additive toxicity results from the simultaneous administration of 21 pairs of organophosphorus insecticides by the intraperitoneal route to female rats. In those cases where the mortality slightly exceeded 50% a second experiment was performed to ascertain whether or not mortality exceeding 50% should be attributed to slight potentiation of toxicity. To obtain this additional information either of two methods was used. In some cases one-half of the LD_{50} of one compound, selected from dose-response curves in which percentage mortality expressed in probits is plotted against the logarithm of the dose, was given in combination with one-half of the LD_{50} of the other compound. The expected mortality was the sum of the expected values for each compound when given singly at these dosage levels, as indicated from the dosage-mortality curves for each compound. The other test which was used for this purpose consisted of the administration of equitoxic doses equivalent to 40% of the LD_{50} of each compound. This test was used in the case of Co-Ral, which exhibited a little more than 50% mortality when given at dosage levels of one-half the LD_{50}

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* Registered trademark Chemagro Corporation, Kansas City, Mo

in combination with Di-Syston, EPN, Guthion, and Diazinon. The administration of 40% of the LD_{50} of Co-Ral plus a corresponding amount of Di-Syston, EPN, Guthion, or Diazinon resulted in 30, 30, 20, and 30% mortalities, respectively, which indicated that no significant amount of potentiation occurs with these combinations.

TABLE III
Additive Acute Toxicity by Various Combinations of Organophosphorus Insecticides[a]

Organophosphate	Second organophosphate	Mortality	Mortality, %
Parathion	EPN	9/20	45
Parathion	Dipterex	11/20	55
Parathion	Systox	10/20	50
Systox	EPN	9/20	45
Systox	Malathion	12/20	60
Guthion	EPN	12/20	60
Co-Ral	Systox	9/20	45
Co-Ral	Di-Syston	12/20	60
Co-Ral	Parathion	10/20	50
Co-Ral	Guthion	12/20	60
Co-Ral	Trithion	10/20	50
Co-Ral	Diazinon	13/20	65
Co-Ral	EPN	13/20	65
Dipterex	Tetram	9/20	45
Dipterex	Phosdrin	8/20	40
Di-Syston	Parathion	11/20	55
Di-Syston	Systox	9/20	45
Di-Syston	Malathion	11/20	55
Di-Syston	EPN	8/20	40
Di-Syston	Trithion	9/20	45
Di-Syston	Diazinon	10/20	50

[a] One-half of the LD_{50} of each compound was given simultaneously to female rats.

All of the pairs of compounds listed in Table III which produced more than 50% mortality in the initial test caused no more than additive acute toxic effects in the second test. Thus, in all of these cases differences of 5–15% from the theoretical expected value can be attributed to experimental variations which were detected by the second experiment. It is conceivable, however, that more exhaustive studies might be necessary in other cases to determine whether or not potentiation occurs with a pair of compounds, particularly if the initial LD_{50} values for one or both of the compounds were inaccurate.

Rosenberg and Coon (13) recently studied the acute toxicity of several pairs of organophosphates in mice. Their index of potentiation was the occurrence of greater mortality by two compounds given simultaneously than was observed with each compound given alone at twice the dose used in the mixture. This is a suitable procedure provided that doses which will produce some mortality are chosen, for each compound, as was the case in this particular investigation. In this connection it is noteworthy that the selection of doses too low to produce mortality when given in the absence of another compound would make this particular type of test less sensitive than the determination of the LD_{50} of equitoxic mixtures or the use of one-half of the LD_{50} of each compound. Among the pairs of anticholinesterase agents tested by these investigators the following did not show definite potentiation: malathion and octamethylpyrophosphoramide, malathion and parathion, malathion and physostigmine, EPN and parathion, EPN and octamethylpyrophosphoramide, and parathion and octamethylpyrophosphoramide. The results obtained with a combination of malathion and *bis*-dimethylamino fluorophosphate (BFP) were considered to be borderline between strict additivity and potentiation. These authors did not distinguish between less than additive and strictly additive toxicity by these pairs of compounds, but the data obtained in this laboratory (Tables III and IV) provide information on this point for most of the same pairs of compounds.

B. LESS THAN ADDITIVE ACUTE TOXICITY BY COMBINATIONS OF ORGANOPHOSPHORUS INSECTICIDES

Less than additive acute toxicity was obtained in this laboratory with 18 pairs of organophosphorus insecticides, as shown by the data in Table IV. In these tests one-half of the LD_{50} of each member of a pair was given simultaneously to female rats. It is noteworthy that these pairs of compounds have the same mechanism of action and that their LD_{50} regression lines are parallel but additional factors prevent the occurrence of strictly additive toxicity. In this connection the time of occurrence of maximal depression of cholinesterase activity by each compound is important. Some organophosphorus compounds must undergo metabolic conversion to active metabolites,

TABLE IV
Less Than Additive Acute Toxicity by Combinations of Organophosphorus Insecticides[a]

Organophosphate	Second organophosphate	Mortality	Mortality, %
Parathion	Malathion	2/20	10
Parathion	Guthion	2/20	10
Dipterex	EPN	6/20	30
Dipterex	Systox	2/20	10
Malathion	Guthion	2/20	10
Systox	Guthion	1/20	5
Co-Ral	Dipterex	6/20	30
Co-Ral	Methyl parathion	6/20	30
Co-Ral	Phosdrin	6/20	30
Co-Ral	Tetram	5/20	25
Di-Syston	Tetram	5/20	25
Dipterex	Systox	3/20	15
Dipterex	Methyl parathion	4/20	20
Dipterex	Trithion	2/20	10
Dipterex	Diazinon	2/20	10
Dipterex	Di-Syston	5/20	25
Di-Syston	Methyl parathion	4/20	20
Di-Syston	Guthion	3/20	15

[a] One-half of the LD_{50} of each compound was given simultaneously to female rats.

and the onset of symptoms depends upon their conversion to active metabolites having anticholinesterase activity. The rate at which this conversion takes place differs for various compounds. Consequently, the time after administration at which each one exerts its maximal toxic effect differs from one compound to another. In addition, the rates of absorption and detoxification are different for various cholinergic organophosphates. Some compounds exhibit rather pronounced differences in one or both of these factors and consequently their simultaneous administration does not result in the occurrence of the maximal toxic effects of each compound at the same time. Thus strict additivity of the toxic actions does not occur. The same type of responses can be expected with combinations of other types of insecticides or chemical agents which have the same mode of action but different rates of absorption and metabolism. It is too often assumed that compounds having the same mode of action will have biological effects approaching strict additivity when given simultaneously. Potentiation tests on organophosphorus insecticides have provided a good illustration of the factors which can prevent additive effects from occurring. It is likely that in our studies on combinations of organophosphates which produced less than additive effects, strict additivity could have been achieved if the time between the administration of each compound was spaced in such a way that the maximal action of the compounds coincided with respect to time of occurrence. This could be accomplished experimentally more readily with organophosphates than with many other groups of chemical agents because the anticholinesterase action of a particular organophosphate *in vivo* can be used as the indicator of the time at which the maximal effect occurs. Otherwise, it is necessary to find this time by testing the toxicity of the two compounds using a variety of intervals between the time of administration of the two agents.

C. POTENTIATION OF ACUTE TOXICITY BY COMBINATIONS OF ORGANOPHOSPHORUS INSECTICIDES

In the studies which have been conducted in this laboratory only 4 of a total of 43 pairs of organophosphorus insecticides which have been tested caused potentiation of acute toxicity. One of these pairs (malathion and EPN) was shown previously by Frawley *et al.* (5,6) to cause potentiation. In addition, one organophosphate (tri-*o*-tolyl phosphate) which is not used as an insecticide was found to potentiate markedly the toxicity of malathion. The data in Table V show that no animals survived the simultaneous intraperitoneal administration of one-half of the LD_{50} of malathion plus the same fraction of the LD_{50} of EPN. Similarly when combinations of Dipterex plus Guthion, malathion plus Dipterex, and Co-Ral plus malathion were given at one-half of the LD_{50} level of each compound, 100% mortality occurred.

To obtain further information concerning the degree of potentation by the pairs of organophosphates which caused more than additive toxicity, the LD_{50} values for equitoxic mixtures of the compounds were determined. Equitoxic mixtures are prepared by mixing the two compounds in amounts proportional to their respective toxicities. Thus, if the LD_{50} values for two compounds are 95 and 5 mg./kg., an equitoxic mixture would contain 95 parts of the first compound

TABLE V
Potentiation of Acute Toxicity by Combinations of Organophosphorus Insecticides[a]

Organophosphate	Second organophosphate	Mortality	Mortality, %
Malathion	EPN	20/20	100
Malathion	Dipterex	20/20	100
Dipterex	Guthion	20/20	100
Malathion	Co-Ral	20/20	100

[a] One-half of the LD_{50} of each compound was given simultaneously to female rats.

and 5 parts of the second. The expected LD_{50} of this mixture would be 50 mg./kg. which represents the sum of one-half of the LD_{50} of each compound. By comparing the ratio of the observed to the expected LD_{50} value obtained with mixtures it is possible to ascertain whether the compounds exert additive or greater than additive toxicity (potentiation). If the numerical ratio of the expected to the observed LD_{50} is greater than one it may be concluded that potentiation of toxicity has occurred. The application of this technique is illustrated in Table VI where it may be seen that the ratios for combinations of malathion and EPN, Dipterex and malathion, Dipterex and Guthion, and Co-Ral and malathion exceeded unity in every case. It is not possible to attribute any particular significance to the numerical degree of potentiation except that it gives some indication of the hazard connected with simultaneous exposure to the two compounds. Thus, when the ratio of expected to observed LD_{50}

TABLE VI
Degree of Potentiation of Acute Toxicity by Combinations of Organophosphates

Organophosphates (equitoxic mixture)	LD_{50}, mg./kg. Expected	LD_{50}, mg./kg. Observed	Ratio of expected LD_{50} to observed LD_{50}
Malathion (99%) plus EPN (1%)	403.6	230	1.8
Dipterex (16.7%) plus malathion (83.3%)	480.0	220	2.2
Dipterex (96.5%) plus Guthion (3.5%)	82.8	55	1.5
Co-Ral (12.1%) plus malathion (87.9%)	455.0	190	2.4

values is two, the toxicity, and consequently the degree of hazard, from exposure to a certain pair of compounds is twice as great as it is when strictly additive toxicity is obtained. However, as will be explained in a subsequent section of this communication, this degree of hazard applies only when the composition of the mixture is that of an equitoxic mixture. When the problem is considered in terms of mechanisms involved, it is clear that the degree of hazard can be much higher than revealed by toxicity tests on equitoxic mixtures of two compounds.

Recent studies by Rosenberg and Coon (13) have indicated that EPN, malathion, Chlorthion, and Phostex in all possible paired combinations produce potentiated toxicity in mice. Potentiation was assumed by these investigators to occur if doses of each compound which produced no mortality when given alone caused greater mortality in combination with another compound than was obtained with twice the dose of either compound alone. Examination of their data indicates that mixtures of EPN and malathion, malathion and Chlorthion, malathion and Phostex, and Chlorthion and Phostex produced considerable potentiation. However, the effects obtained with EPN plus Chlorthion and EPN plus Phostex were rather small. These findings add a substantial number of potentiating pairs of organophosphates to the number observed by other investigators. It brings the incidence of potentiation to 20% of the combinations which have been examined and reported in this and other articles in the scientific literature. On the basis of this rather high percentage it must be concluded that potentiation of the acute mammalian toxicity of organophosphorus insecticides is a toxicological problem which must not be minimized. It is clear from these findings that every precaution should be taken to avoid simultaneous acute exposures to some pairs of organophosphates during their manufacture and practical application. In the absence of information on the possibility that a pair of compounds causes potentiation of toxicity it seems reasonable to allow a generous margin of safety in the precautions recommended for the safe handling of these agents.

D. POTENTIATION OF TOXICITY OF MALATHION BY TRI-*o*-TOLYL PHOSPHATE

In Section V evidence is presented which indicates that potentiation of the toxicity of malathion by EPN is due to the ability of the latter compound to inhibit the enzymic detoxification of malathion. Since this detoxification process is catalyzed by nonspecific esterases, it seemed likely that any compound capable of inhibiting the detoxification enzymes would potentiate the toxicity of malathion. If this were the case, the problem of potentiation of the toxicity of organophosphorus insecticides would not be limited to various combinations of insecticides, but would extend far beyond this group of compounds to include drugs, food additives, and industrial chemicals. In connection with this possibility Murphy, Anderson, and DuBois (7) tested the toxicity of tri-*o*-tolyl phosphate (TOTP) in combination with malathion. TOTP is not used as an insecticide at the present time, but has other important industrial uses, including employment as a plasticizer. The testing of this compound as a possible potentiating agent was suggested by the previous finding of Myers and Mendel (11), that tri-*o*-tolyl phosphate inhibits nonspecific esterases of the liver and serum of rats.

Prior to use for potentiation tests, the acute toxicity of TOTP to rats was determined. The LD_{50} to rats was 2500 mg./kg. when the compound was given intraperitoneally to female rats. Myers and Mendel (11) found that intramuscular injection of 0.1 ml. of TOTP on alternate days for 5 days caused 97% inhibition of the nonspecific esterase activity of liver and serum of rats. Our initial experiments on the influence of TOTP on the toxicity of malathion consisted of administration of three intramuscular doses of 440 mg./kg. each on alternate days, followed by one-half of the LD_{50} (550 mg./kg.) of malathion given intraperitoneally 24 hours after the last dose of TOTP. All animals given this treatment died within 1 hour after malathion, whereas no mortality occurred in controls which received either drug alone. When three intramuscular doses of 110 mg./kg. were administered on alternate days, the intraperitoneal administration of 550 mg./kg. of malathion caused 100% mortality when given 24 hours after the last dose of TOTP. When the dose of TOTP was reduced to 10 mg./kg., death of all of a group of five rats given 550 mg./kg. of malathion occurred. The particular solvent used for TOTP and malathion was not an important factor in producing the potentiation, since TOTP (110 mg./kg.) given in the undiluted form or as sesame oil or propylene glycol solutions caused 100% mortality of rats given 550 mg./kg. of malathion 24 hours later.

Following these initial experiments a more extensive study was made of the effect of TOTP on susceptibility of rats to the acute toxic effects of malathion. Various routes, doses, and dosage schedules were used for TOTP, since industrial exposure to a combination of agents which causes potentiation might be expected to occur under a variety of exposure conditions. The results of these experiments are summarized in Table VII. Potentiation of toxicity occurred regardless of the route of administration of TOTP. The greatest increase in susceptibility was noted when malathion was given 24 hours after the intraperitoneal or cutaneous administration of TOTP. The greater toxicity of malathion to rats given TOTP 24 hours earlier as compared with simultaneous administration of the two agents indicated that the relative time of administration of the two compounds is an important factor in determining the degree of potentiation. It is probable that the degree of potentiation exhibited by various pairs of insecticidal organophosphates is likewise influenced by the interval between exposure to the two compounds, but this aspect of the problem has thus far received essentially no attention.

TABLE VII
Effect of TOTP on Susceptibility of Rats to Malathion

TOTP dosage and route of administration	Time of administration of malathion[a]	Number of animals	Approximate LD_{50} of malathion, mg./kg.
No TOTP		32	1100
10 mg./kg.; intramuscular on alternate days for 5 days	24 hrs. after last inj. of TOTP	25	125
110 mg./kg.; cutaneous, single application	24 hrs. after TOTP	34	12.5
110 mg./kg.; intraperitoneal	24 hrs. after TOTP	28	8.2
110 mg./kg.; intraperitoneal	Simultaneously with TOTP	22	61
10 mg./kg.; intraperitoneal	Simultaneously with TOTP	25	175

[a] Malathion administered intraperitoneally in all cases.

After simultaneous administration of malathion and TOTP deaths usually occurred within 30–60 minutes after administration of the combination, which corresponds to the time of death of animals given large doses of malathion alone. None of the animals treated with TOTP exhibited any untoward effects until malathion was administered. These observations indicated that the only significant action of TOTP was to increase the susceptibility of rats to malathion. Further evidence in support of this idea was obtained by experiments in which the sequence of administration of malathion and TOTP was varied. When 110 mg./kg. of TOTP was given intraperitoneally to male rats 24 hours before injection of 550 mg./kg. of malathion, symptons of malathion poisoning developed rapidly and death of all animals occurred. In contrast, when malathion was administered 24 hours before TOTP, no symptoms or mortality were observed. Evidence that the potentiating action of TOTP is of long duration was furnished by experiments in which a dose of 550 mg./kg. of malathion was given to four groups each containing five rats at 1, 3, 7, and 14 days after a single intraperitoneal dose of 110 mg./kg. of TOTP. One hundred per cent mortality occurred in all groups within 1 hour after administration of malathion.

The results of this particular investigation demonstrated that the toxicity of malathion to rats is markedly enhanced by prior or simultaneous administration of TOTP, and additional experiments which are described in Section V indicate that the potentiation of toxicity is due to inhibition by TOTP of the enzymic detoxification of malathion. It is clear from the results of this study that the scope of the problem of potentiation of the toxicity of organophosphates extends beyond the possible interference by one insecticidal organophosphate with the metabolism of another compound having a similar chemical structure and use. A complete evaluation of the potential health hazards which might arise as a result of potentiation of the toxicity of organophosphorus insecticides would require consideration of drugs, industrial chemicals, food additives, and other chemical agents which commonly gain entrance to the body. This aspect of the problem of potentiation of the toxicity of insecticides has been discussed in a recent article by DuBois (3). The problem is further complicated by the fact that a potentiating agent such as tri-o-tolyl phosphate will inhibit the detoxification of malathion at dosage levels of the potentiating agent which produce no detectable toxic effects. The use of toxicity tests for detection of potentiation by the numerous possible combinations of chemical agents which can be ingested simultaneously is not possible from a practical standpoint. However, extension of our present knowledge of the mechanisms by which certain compounds act as potentiating agents should greatly facilitate detection of combinations of insecticides and other agents which cause potentiation of toxicity.

IV. Use of Subacute Toxicity Tests for Detecting Potentiation by Organophosphorus Insecticides

The importance of potentiation of the toxicity of organophosphorus insecticides to the consumer of foods treated with these chemicals cannot be fully evaluated by the use of acute toxicity tests. Such tests demonstrate whether or not a particular pair of compounds is capable of causing potentiated toxicity and thus provide a convenient means of eliminating from further consideration those combinations which are incapable of causing potentiation. This statement is based on the present concept that potentiation of the toxicity of organophosphorus insecticides is the result of inhibition by one compound of the enzymic detoxification of the other compound. With this mechanism in mind, it is apparent that compounds which are unable to inhibit the detoxifying enzymes when given at the high doses used for acute toxicity tests are likewise unable to affect detoxification mechanisms at the low levels which are permitted as food contaminants. Thus consideration of the hazards from potentiation of toxicity to the health of consumers of contaminated food crops can be limited to those combinations which cause definite acute potentiation. The practical problem then becomes one of determining whether a particular compound has sufficient potency as an enzyme inhibitor at low dietary levels to interfere with the normal detoxification of other insecticides. Subacute tests on dogs have been adopted as the standard method for determining whether various pairs of organophosphorus insecticides capable of causing acute potentiation can produce this effect when fed in the diet. Since no mortality results from feeding the tolerance levels of any combination of organophosphates tested thus far, potentiation of toxicity is measured by the performance of blood cholinesterase measurements. The occurrence of potentiation is readily detected by a decrease in blood cholinesterase levels which markedly exceeds the decrease that results from simple addition of the anticholinesterase action of individual compounds.

All of the pairs of organophosphorus insecticides which cause potentiation of acute toxicity in rats (Table VI) have been subjected to the subacute tests by feeding various dietary levels to dogs. The combination of EPN and malathion has been studied most extensively beginning with the initial experiments of Frawley *et al.* (5,6), who fed diets containing various levels of EPN and malathion to dogs and rats. They found that the feeding of diets containing 250 p.p.m. of malathion and 50 p.p.m. of EPN to dogs causes up to 60% inhibition of plasma cholinesterase and 93% inhibition of erythrocyte cholinesterase activity. A diet containing 100 p.p.m. of malathion and 20 p.p.m. of EPN caused only questionable inhibition of plasma cholinesterase but up to 68% inhibition of erythrocyte activity. It is noteworthy that these are the maximum dietary levels of each compound (safe levels) which produce no inhibition of tissue cholinesterase when fed separately. A combination of the tolerance levels (8 p.p.m. of malathion and 3 p.p.m. of EPN) caused up to 24% inhibition of erythrocyte cholinesterase in 8 weeks, but failure of the activity to return to the pretreatment control levels when the diet was withdrawn suggests that this depression was not due to potentiation. Similar experiments with rats indicated that a diet containing 100 p.p.m. of malathion and 5 p.p.m. of EPN caused no more than additive inhibition of the cholinesterase activity of rat blood, whereas 500 p.p.m. of malathion and 25 p.p.m. of EPN caused 21% inhibition of the enzyme activity of whole blood. Doull and Vaughn (2) reinvestigated the effects of this combination using dietary levels of 20 p.p.m. of EPN and 100 p.p.m. of malathion. They found 56% inhibition of erythrocyte cholinesterase and 27% inhibition of serum cholinesterase activity in dogs during a 6 week observation period, which is in general agreement with the findings of Frawley *et al.* (5,6). However, when rats were fed these same levels, 76% inhibition of erythrocyte cholinesterase and 56% inhibition of the plasma enzyme were observed, which is a somewhat greater effect than was obtained by the previous investigators (5,6). When the results of these experiments are interpreted in terms of the exact mechanism responsible for potentiation (1,9), it is clear that the dietary level of EPN is the critical factor which determines the amount of potentiation that can occur, since this compound inhibits the detoxification of malathion. If sufficient EPN is present in the diet to inhibit effectively the enzyme that detoxifies malathion, then the depression of enzyme activity is increased to an extent which is dependent upon the amount of malathion in the diet.

Since the potentiating effects of EPN and malathion occurred in experimental animals at dietary levels extremely close to those currently permitted on food crops, the question of whether these levels were safe for man attracted considerable interest. A study was, therefore, conducted by Rider (12) in which the tolerance levels (3 mg. of EPN and 8 mg. of malathion per man) were fed daily to healthy male subjects for 6 weeks. No depression of plasma or red cell cholinesterase activity was observed. However, when daily doses of 6 mg. of EPN plus 16 mg. of malathion were fed, some depression of enzyme activity was observed, but it was not large enough to be interpreted as potentiation (12). Thus it appears that this combination does not present a health hazard in man and the dangers associated with potentiation by this pair of compounds are limited to dosage levels above the maximum permissible dietary levels of 3 mg. of EPN and 8 mg. of malathion.

The potentiation of toxicity which occurred with combinations of Dipterex plus malathion and Dipterex plus Guthion, as demonstrated by acute toxicity experiments (Table VI), stimulated the interest of Doull and Vaughn (2) in determining whether potentiation occurred when the compounds were fed to dogs. For these experiments diets containing 100 p.p.m. of Dipterex plus 5 p.p.m. of Guthion and 100 p.p.m. of Dipterex plus 100 p.p.m. of malathion were fed to dogs for a period of 6 weeks. These are the maximum dietary levels which produce no inhibition of cholinesterase activity when the compounds are fed separately; the levels permitted on food (tolerance levels) would be considerably lower in every case. Thus, the absence of potentiation under the conditions of these experiments is assurance that potentiation of toxicity will not occur at the tolerance levels. Feeding the combination of Dipterex and Guthion to dogs resulted in an average of 18% inhibition of erythrocyte cholinesterase and 15% inhibition of plasma esterase, whereas the enzyme activity of the red cells and plasma was 38% inhibited in rats. Since up to 40% inhibition can be expected from additive effects of two organophosphates fed at the safe levels, it was concluded that potentiation of the toxicity of this combination did not occur under these conditions and no hazard would be involved in the consumption of food contaminated with permitted levels of these compounds.

Similarly, the feeding of diets containing Dipterex (100 p.p.m.) plus malathion (100 p.p.m.) caused no potentiation in dogs as evidenced by 33 and 35% inhibition of the cholinesterase activity of erythrocytes and serum. In rats fed the same levels a maximum of 34% inhibition of erythrocyte cholinesterase activity and 48% inhibition of the serum enzyme was noted. The effect on serum appeared to be at the borderline of potentiation.

Doull and Vaughn (2) also determined whether the strong potentiation which results from the simultaneous administration of acutely toxic doses of malathion and Co-Ral occurred when this combination was fed to dogs. The safe level of Co-Ral was found to be 2 p.p.m. for dogs. When this level was fed in combination with 100 p.p.m. of malathion, it produced 40% inhibition of the cholinesterase activity of serum and erythrocytes. This amount of inhibition is at the level which must be attributed to additive effects rather than potentiation.

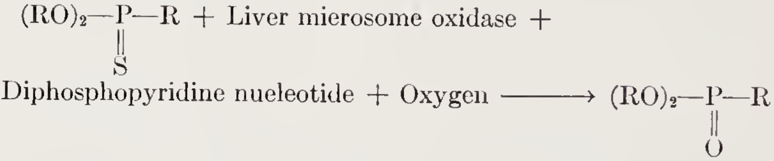
It is clear from the results of these experiments that the combinations of organophosphorus insecticides which produce potentiation of acute toxicity do not exhibit this effect when fed at the low dietary levels permitted in food. On the basis of the data presented in Section V it may be concluded that the compound which inhibits the enzyme(s) that catalyzes the detoxification of the other compound in each of these potentiating pairs is not a sufficiently active inhibitor at the levels fed in the diets to cause any increase in toxicity. However, it is worthy of emphasis that partial inhibition of the detoxifying enzymes, though constituting a biochemical injury, does not affect the ability of animals to detoxify low dietary levels of organophosphates. Thus, the most accurate way of determining the effects of potentiating agents is not to measure the end result in terms of inhibition of cholinesterase but rather to use a direct assay of the activity of the detoxifying enzymes in the tissues of animals fed organophosphates.

V. Mechanisms Responsible for Potentiation of Toxicity of Organophosphorus Compounds

The performance of acute and subacute toxicity tests on combinations of organophosphorus insecticides is the most reliable method of determining the possible health hazards from potentiation. However, the interpretation of toxicity data is difficult without information on the mechanism responsible for the observed toxic effects. Whether one agent potentiates the toxicity of the other compound cannot be readily ascertained from toxicity studies alone. The exact mechanisms responsible for potentiation of the toxicity of all of the various pairs which exhibit this effect are not yet understood, but progress has been made in elucidation of the reasons for potentiation of EPN plus malathion, Dipterex plus malathion, and tri-*o*-tolyl phosphate plus malathion.

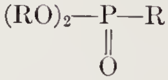
In connection with the possible mechanisms responsible for potentiation of the toxicity of organophosphorus insecticides three enzymic reactions seemed worthy of consideration, namely, the activation, mechanism, and metabolism of these compounds. These processes involve the following types of reactions:

1. *Activation:*



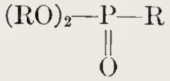
$$(RO)_2—\underset{\underset{\displaystyle S}{\|}}{P}—R + \text{Liver microsome oxidase} + \text{Diphosphopyridine nucleotide} + \text{Oxygen} \longrightarrow (RO)_2—\underset{\underset{\displaystyle O}{\|}}{P}—R$$

2. *Mechanism of toxic action:* Inhibition of cholinesterase by



$$(RO)_2—\underset{\underset{\displaystyle O}{\|}}{P}—R$$

3. *Detoxification:* Hydrolysis of



$$(RO)_2—\underset{\underset{\displaystyle O}{\|}}{P}—R$$

catalyzed by aliesterases of liver, serum, and other tissues.

The first step in the metabolism of thiophosphates consists of oxidative removal of the sulfur atom and its replacement by oxygen. This reaction is catalyzed by a pyridine nucleotide–linked enzyme in liver microsomes. Thiophosphates are inactive as anticholinesterase agents but are converted by this enzyme system into highly toxic anticholinesterase agents. For this reaction to be involved as the cause of the potentiation, one thiophosphate would have to accelerate the conversion of the other compound to its corresponding oxygen analogue. The recent development in this laboratory (9) of a quantitative method for measuring the rate of conversion of thiophosphates to their corresponding oxygen analogues facilitated the testing of this possibility. The application of this method to the case of EPN and malathion showed that neither compound interfered with the conversion of the other one to an anticholinesterase agent, thus indicating that potentiation of the toxicity of these agents does not involve acceleration by one compound of the enzymic conversion of the other agent to its oxygen analogue.

A second possible mechanism, which seemed unlikely, was a direct increase in anticholinesterase activity of one compound by the presence of the other compound. However, the oxygen analogues of EPN and malathion in combination produced strictly additive effects on cholinesterase *in vitro.*

After elimination of two of the possible mechanisms responsible for potentiation of the toxicity of organophosphates, attention was focused on the possible interference by one compound with the detoxification of the other one. Direct evidence in support of this possibility was obtained by Cook, Blake, and Williams (1). They found that malathion is altered very rapidly by an esterase in liver and that the detoxification consists of hydrolysis of either or both ester linkages of the 1,2-dicarbethoxyethyl side chains. These investigators found that a number of organophosphates inhibited the hydrolysis of malathion *in vitro,* but only Diazinon was more effective than EPN. However, it appears that *in vitro* tests are considerably more sensitive as indicators of the potentiating ability of a particular pair of organophosphates than are toxicity measurements. Furthermore, a particular compound can be tested *in vitro* at concentrations much higher than those which can be achieved in the intact animal because of the high toxicity of some of the organophosphorus insecticides. Thus, caution must be exerted in the interpretation of *in vitro* results in terms of possible effects in the intact animal.

In undertaking studies on the mechanism of potentiation of the toxicity of malathion by EPN in this laboratory efforts were directed toward the development of a quantitative method for measuring the inhibitory effect of EPN on the detoxification of malathion. For this approach a quantitative procedure was needed for measuring the rate of enzymic detoxification of malathion and its oxygen analogue (malaoxon) in animal tissues. A number of preliminary experiments on the optimum conditions for measuring the activity of the esterase resulted in the development of a procedure which satisfied the requirements of a quantitative enzyme assay. The oxygen analogue of malathion was employed as the substrate in the test system, since it has strong anticholinesterase activity in contrast to pure malathion which has no action on this enzyme *in vitro.* It was possible

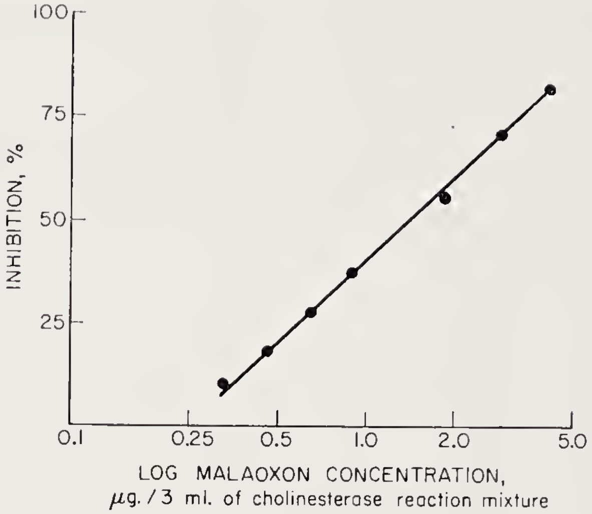


Fig. 1. Inhibitory effect of malaoxon on brain cholinesterase *in vitro.* (From Murphy and DuBois, 9)

to utilize the anticholinesterase activity of the oxygen analogue (malaoxon) to bioassay the quantity of malaoxon destroyed by the esterases of animal tissues. The test system contained 0.15 ml. of 5 × 10^{-4} *M* malaoxon, 0.4 ml. of 0.1 *M* phosphate buffer (pH 7.2), 0.1 or 0.2 ml. of cold, aqueous tissue homogenate, and enough distilled water to make a final volume of 3 ml. Assays were performed in duplicate using two levels of tissue. Thus, 0.1 and 0.2 ml. of 2.5% liver (2.5 mg. and 5.0 mg.) and 10 and 20 mg. of serum were used except in those experiments in which the inhibitory effect of EPN necessitated the use of higher quantities of tissue. The tubes containing all of the constituents of the reaction mixture except the substrate were placed in a constant temperature bath at 38°C., and the malaoxon was then added. After incubation for 10 minutes aliquots (0.6 ml.) were withdrawn and added immediately to the cholinesterase test system containing 50 mg. of homogenized rat brain as the source of the enzyme. From the amount of inhibition of cholinesterase produced by malaoxon before and after incubation with tissue homogenates, it was possible to calculate the quantity of malaoxon which was detoxified using the logarithmic relationship between inhibition and malaoxon concentration in Figure 1.

In the test system described above the rate of detoxification of malaoxon was dependent upon the tissue concentration. Thus

2.5 and 5 mg. of liver detoxified 9.1 and 20.1 μg. of malaoxon, respectively. Under the conditions selected for the assay the reaction rate was linear with time, as evidenced by detoxification of 4.7, 8.9, and 13.7 μg. of malaoxon by 2.5 mg. of liver in 5, 10, and 15 minutes, respectively. Liver homogenates heated at 100°C. for 2 minutes lost their ability to catalyze the detoxification of malaoxon; dialysis against distilled water for 18 hours at 5°C. caused only 6% loss of enzyme activity. Storage of whole liver homogenates at 5°C. for 18 hours caused only 10% loss of activity.

The first application of the method for measuring the detoxification of malaoxon consisted of a survey of the enzyme activity of tissues from various mammalian species. The results of these measurements are summarized in Table VIII in which each value is an average of assays on the tissues of at least three animals. These measurements demonstrated that liver exhibits the highest activity in all species used, with dog liver being the most active. The serum of mice and rats was capable of detoxifying a considerable quantity of malaoxon, but dog serum exhibited no activity. Kidney and lung also contained the detoxifying enzyme. Other assays conducted on female mice and rats indicated that there is no sex difference in the enzyme activity of the liver of mice and the serum of mice and rats. However, the activity of the liver of male rats was about four times as great as that of females as evidenced by the detoxification of 4 (range of 3.4–4.5) μg. of malaoxon/mg. of male rat liver and 0.99 (range of 0.9–1.1) μg./mg. of female rat liver.

TABLE VIII
Detoxification of Malaoxon by Tissues of Various Species (9)
(μg. of malaoxon destroyed/mg. of tissue/10 minutes)

Tissue	Rat	Mouse	Guinea pig	Dog
Liver	4.03 ± 0.17	1.84 ± 0.15	3.74 ± 0.28	11.5 ± 1.1
Kidney	0.66 ± 0.04	0.46 ± 0.05	0.21 ± 0.13	1.85 ± 0.66
Serum	0.99 ± 0.07	1.78 ± 0.08	0.19 ± 0.03	0.08 ± 0.06
Lung	0.75 ± 0.05	0.58 ± 0.02	0.19 ± 0.13	1.18 ± 0.11
Ileum	0.39 ± 0.02	0.23 ± 0.03	0.13 ± 0.04	0.07 ± 0.02
Spleen	0.12 ± 0.03	0.03 ± 0.01	0.16 ± 0.12	0.03 ± 0.03

Addition of EPN to the enzyme system which catalyzes the detoxification of malaoxon markedly inhibited the reaction. When various

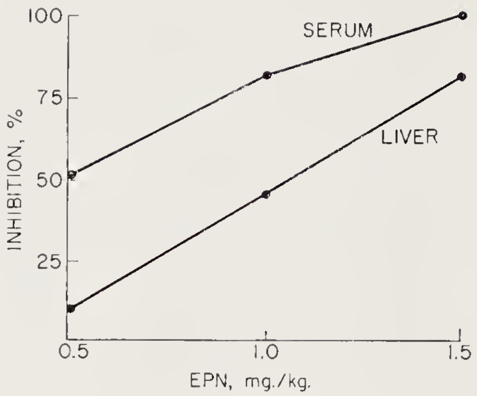


Fig. 2. Inhibitory effect of EPN *in vivo* on the ability of rat tissues to detoxify malaoxon. Assays performed on tissues taken from rats 1 hour after intraperitoneal administration of EPN. (From Murphy and DuBois, 9)

concentrations of EPN were incubated with homogenized liver for 5 minutes prior to addition of malaoxon, the amount of inhibition of the detoxifying esterase was proportional to the logarithm of the EPN concentration within the range of EPN concentrations producing between 20 and 80% inhibition. In these tests 50% inhibition of the malathion-detoxifying enzyme activity of male rat liver occurred with a final concentration of 2.8×10^{-5} *M* EPN, and the lower activity of the liver of female rats was inhibited to the same extent by 5×10^{-6} *M* EPN. To ascertain whether this inhibitory effect also occurs *in vivo* a group of three adult male rats was given one-half of the LD_{50} of EPN (13 mg./kg.) intraperitoneally, and 1 hour later the animals were sacrificed for measurements of the enzyme activity of serum and liver. Complete inhibition of the ability of the serum and liver to detoxify malaoxon was observed. This finding was followed by similar experiments in which several lower doses of EPN were tested to find the quantity which produced partial inhibition of the detoxification enzyme. The results summarized in Figure 2 represent the average of assays performed on tissues from groups each containing at least three male rats which received 0.5, 1, or 1.5 mg./kg. of EPN intraperitoneally. The per cent inhibition of enzyme activity was calculated from the assay results using the normal values shown in Table VIII as a basis for the calculations. The data in Figure 2 show that 84 and 93% inhibition of the ability of liver and serum of male rats to detoxify malaoxon occurred in 1 hour after the administration of 1.5 mg./kg. of EPN. Significant

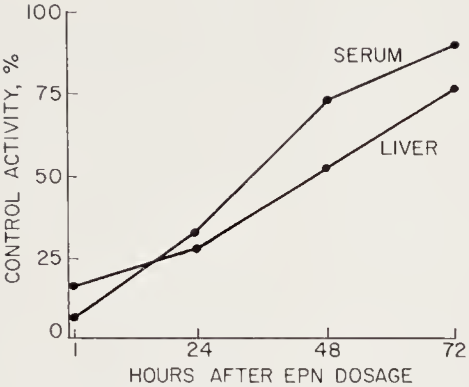


Fig. 3. Duration of the inhibitory effect of a single intraperitoneal dose of EPN (1.5 mg./kg) on the ability of rat tissues to detoxify malaoxon. (From Murphy and DuBois, 9)

inhibition was also observed with 0.5 mg./kg. of EPN, which is approximately one fiftieth of the LD_{50}. The effect of inhibition of the detoxifying esterase by EPN on the susceptibility of rats to the acute toxicity of malathion was demonstrated by determining the LD_{50} of malathion to male rats treated 1 hour previously with 1.5 mg./kg. of EPN. The approximate LD_{50} of malathion to these animals was 550 mg./kg. as compared with 1100 mg./kg. for comparable controls. The dose of EPN used in this experiment did not cause any inhibition of the cholinesterase activity of brain, serum, and submaxillary glands, and the increased toxicity of malathion can, therefore, be attributed solely to inhibition of its normal detoxification.

The duration of the inhibitory effect of EPN was studied by administering 1.5 mg./kg. of EPN to a series of male rats and sacrificing them at various intervals for assays on serum and liver. The results of these measurements are shown in Figure 3, in which each value on the curves represents an average for the tissues of three animals. Slow reversal of the enzyme inhibition was observed as evidenced by a gradual return of the activity during a 72 hour observation period.

It was considered important to ascertain the effects of diets containing EPN on the activity of the enzyme which detoxifies malaoxon, since EPN is used as an insecticide on food crops and has a tolerance established at 3 p.p.m. For this experiment groups each containing four male rats were fed various levels of EPN in the diet for 2 weeks and were then sacrificed for measurements of the ability of liver and

TABLE IX

Effect of Feeding EPN on the Ability of Rat Tissues to Detoxify Malaoxon (9)

Dietary level of EPN, p.p.m.	Malaoxon destroyed/mg. of tissue/10 min., µg.		Inhibition, %	
	Liver	Serum	Liver	Serum
Control	5.6 ± 0.72	1.52 ± 0.23		
5	4.0 ± 0.15	0.92 ± 0.10	28.6	39.5
10	2.4 ± 0.5	0.62 ± 0.18	57.1	59.2
20	1.2 ± 0.15	0.29 ± 0.12	78.6	80.9
50	0.62 ± 0.09	0.13 ± 0.02	87.9	91.4
100	0.28 ± 0.13	0.07 ± 0.03	95.0	95.4

serum to detoxify malaoxon. The results of these assays are summarized in Table IX, where it may be seen that the levels as low as 5 p.p.m. of EPN caused inhibition of the detoxification enzyme. The amount of inhibition was dependent upon the dietary level of EPN over the range of 5–100 p.p.m. of EPN, with nearly complete inhibition being observed at the highest level.

The inhibitory action of EPN on the detoxification of certain cholinergic organophosphates has a useful application in research on the enzymic conversion of thiophosphates to their corresponding oxygen analogues. In a previous communication we described experiments which demonstrated that the enzymic conversion of thiophosphates to their oxygen analogues *in vitro* is complicated by coincident hydrolytic destruction of the compounds and their active metabolites. The strong inhibitory action of EPN on the detoxification of malathion suggested the use of EPN to inhibit destruction during studies on the enzymic conversion of malathion to malaoxon by liver homogenates. When malathion (1×10^{-4} M) was incubated with 2.5–25 mg. of whole liver homogenate, using the test system of Murphy and DuBois (9), no accumulation of malaoxon occurred in the reaction mixture. However, homogenates prepared from the livers of five male rats which were sacrificed at 1 hour after intraperitoneal administration of 13 mg./kg. of EPN produced an average of 1 µg. of malaoxon/mg. of liver/10 minutes. The results of this experiment demonstrated that the conversion of malathion to its cholinergic oxygen analogue can be studied *in vitro* using whole liver homogenates by inhibiting the enzyme responsible for the breakdown of the parent compound and its active metabolite with EPN. It seems likely that this same procedure can be employed for studying the metabolic conversion of some of the other insecticidal thiophosphates to toxic metabolites.

The mechanism of the potentiating action of Dipterex in combination with malathion has also been studied in this laboratory, using the same procedure employed for the experiments with EPN. In undertaking these experiments it was assumed that Dipterex inhibited the detoxification of malathion. Measurements of the inhibitory effect of Dipterex on the ability of animal tissues to detoxify malathion were made by administering various doses of Dipterex intraperitoneally to adult male rats. Each dosage level was given to groups containing three rats. One hour after treatment with Dipterex the animals were sacrificed, and the ability of the tissues to detoxify malathion was measured by the method described above. The results of these measurements are summarized in Table X.

TABLE X
Effect of Various Doses of Dipterex on the Detoxification of Malathion by Rat Tissues

Dose of Dipterex, mg./kg.	Malaoxon destroyed/mg. of tissue/10 min., μg.		Inhibition, %	
	Liver	Serum	Liver	Serum
Control	4.0 ± 0.15	1.43 ± 0.13		
10	2.4 ± 0.5	0.49 ± 0.05	40.0	65.7
20	1.5 ± 0.17	0.42 ± 0.21	62.5	70.1
40	1.2 ± 0.19	0.10 ± 0.06	70.0	93.0

As indicated by the data in Table X, Dipterex is an effective inhibitor of the detoxification of malathion. The LD_{50} of the sample of Dipterex employed for this experiment was 160 mg./kg. Thus, it is clear that low fractions of the LD_{50} are capable of inhibiting the detoxification enzyme. The results of this experiment offer an explanation for the potentiation of toxicity observed in the acute toxicity tests with malathion plus Dipterex.

The duration of the inhibitory effect of Dipterex on the detoxification of malathion was studied by administration of a single dose of 40 mg./kg. of Dipterex and sacrificing groups of three animals at various intervals for measurements of the ability of their tissues to detoxify malathion. These measurements showed that the inhibitory effect of Dipterex is rapidly reversible, in contrast to the more persistent inhibition which was observed with EPN.

Subacute tests performed in this laboratory showed that the safe levels of Dipterex (100 p.p.m.) and malathion (100 p.p.m.) caused no potentiation when fed in the diet to dogs. Further evidence in support of this conclusion was obtained by feeding rats 100 p.p.m. of Dipterex for 2 weeks. The animals were then sacrificed for measurements of the ability of their tissues to detoxify malathion. No inhibition of the malathion-detoxifying enzyme was noted, in confirmation of the results of subacute feeding tests with a combination of the two agents which provided no evidence of potentiation at these dietary levels. It is clear from the results of these experiments that Dipterex is a less potent inhibitor than EPN of the esterase that catalyzes the detoxification of malathion.

The existing evidence that EPN potentiates the toxicity of malathion by inhibiting its detoxifiatcion suggested that a similar mechanism was responsible for the potentiation of acute toxicity that was observed with a combination of malathion and tri-o-tolyl phosphate (TOTP), particularly since the latter compound is a strong inhibitor of nonspecific esterases (11). This possibility was tested by measuring the effect of TOTP on the enzymic detoxification of malathion, using the method of Murphy and DuBois (9). For this experiment three male rats were given 110 mg./kg. of TOTP intraperitoneally. The animals were sacrificed 30 minutes later, and liver and serum were assayed for their ability to detoxify malaoxon. It was found that the liver and serum of TOTP-treated animals detoxified an average of 0.48 and 0.2 μg. of malaoxon/mg. of tissue/10 minutes, respectively, as compared with 3 μg. for the liver and 1.3 μg. for the serum obtained from control animals. The results of this experiment indicated that TOTP increases susceptibility of rats to malathion by inhibiting the esterases responsible for its detoxification. As a result of this inhibitory effect by TOTP a given dose of malathion causes a much greater inhibition of cholinesterase than it normally does. This was demonstrated by giving rats an intraperitoneal dose of 110 mg./kg. of TOTP followed immediately by 150 mg./kg. of malathion. The animals were sacrificed 30 minutes after treatment, together with controls which received each drug alone, and cholinesterase assays were performed on the brain, submaxillary

TABLE XI
Effect of TOTP and Malathion Separately and in Combination on Cholinesterase Activity of Rat Tissues
(in per cent of control activity)

Compound (dose)	Brain	Submaxillary gland	Serum
TOTP (110 mg./kg.)	100	91.2	81.1
Malathion (150 mg./kg.)	100	97.2	67.6
TOTP (110 mg./kg.) plus malathion (150 mg./kg.)	40.3	49.8	40.1

gland, and serum. The results of these measurements are shown in Table XI where it may be seen that the cholinesterase activity was not appreciably affected by malathion or TOTP at the dose levels used for this experiment when the compounds were given separately. However, simultaneous administration of both compounds at the same dosage levels resulted in marked inhibition of the cholinesterase activity of all of the tissues.

Rosenberg and Coon (13) have studied the mechanism of potentiation by those pairs of organophosphorus insecticides which exhibited more than additive acute toxicity. They incubated combinations of compounds with liver slices and measured the anticholinesterase activity to obtain an indication of inhibition of detoxification. These investigators observed that the incubation medium had a higher concentration of anticholinesterase compounds when EPN and malathion, EPN and Chlorthion, EPN and Phostex, and malathion and Chlorthion were present in pairs with liver slices than was observed with the compounds by themselves. These experiments are complicated by the fact that they really dealt with the cholinesterase inactivating impurities present, or the oxygen analogues formed during the incubation of the compounds with liver slices, since neither member of most of these pairs should inhibit cholinesterase, at the concentrations used, until they are converted to their corresponding oxygen analogues. The results obtained with the compounds individually show very little anticholinesterase activity. Thus conversion of the compounds to the oxygen analogues and detoxification must have occurred simultaneously in these experiments. Nevertheless, the results indicate that inhibition of the breakdown of the anticholinesterase agents occurred under the conditions of these experiments, and they provided further evidence that potentiation of the toxicity of organophosphates is due to inhibition of their normal inactivation.

VI. Summary

The initial demonstration by Frawley *et al.* (5,6) of potentiation of toxicity in mammals by a combination of EPN and malathion has been followed by rather extensive studies on various combinations of organophosphorus insecticides. Tests for potentiation are now part of the required toxicological studies on new insecticides with anticholinesterase activity. The present communication describes the results of tests on about 50 pairs of compounds. There is undoubtedly a considerable amount of additional information which has not been published in the scientific literature. Acute toxicity tests have indicated that about 20% of the number tested exhibit some potentiation of toxicity in rats. Whether more or fewer pairs would produce this effect in other species is unknown at the present time. The greatest danger from potentiation of the toxicity of organophosphorus insecticides lies in the possibility of acute exposure to potentiating pairs during their manufacture and application. None of the combinations which cause potentiation acutely produce more than additive toxicity when fed in the diet at the established or proposed tolerance levels. Thus, there is no evidence to date that there is any potential health hazard to the consumer of food upon which potentiating combinations of organophosphorus insecticides have been used, as long as the tolerance levels have not been exceeded. However, evidence is accumulating which indicates that certain chemicals other than insecticides have the ability to inhibit the detoxification of organophosphorus insecticides and consequently potentiate their toxicity. In order to evaluate the extent of the health hazards associated with this aspect of the problem of potentiation, a considerable amount of additional research is needed.

The mechanism responsible for potentiation of the acute toxicity of organophosphorus insecticides involves inhibition of the metabolism of one compound by the other member of a pair. A sufficient number of examples of inhibition of the detoxification of malathion and other compounds by various organophosphates have been established to make this mechanism seem reasonable as an explanation for all cases of potentiation of the toxicity of compounds of this class.

Inhibition of detoxification enzymes by organophosphates constitutes a biochemical injury. In some cases these enzymes are more sensitive to inhibition than is cholinesterase and the tolerance levels for agents such as EPN should be revised accordingly to take this new information into account. Since the detoxification mechanisms for organophosphates might be expected to differ qualitatively and perhaps quantitatively in various species, it seems likely that a great deal of information could be derived by extending the available information to other mammals and to various insect species.

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CHEMICAL CONTROL OF INSECT PESTS OF DOMESTIC ANIMALS

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I. Introduction

Domestic animals, like their wild brethren, are ideal sources of nutriment and habitat for numerous insects and other arthropods. From a parasitological standpoint, insects, mites, and ticks form a group with so many similarities of host relationship, and so many differences from other parasites and predators of domestic animals, that they may appropriately be considered together. Accordingly, the title of this review should perhaps have made reference to arthropod pests rather than to insect pests, since the intent is to include the mites and ticks. In this connection the ecological similarities, rather than the genetic differences, among these arthropods will be stressed, although genetic differences cannot be ignored when the physiological responses to pesticidal chemicals are considered.

Since most of the principles and many of the chemicals to be discussed will concern insects as well as mites and ticks, a little-used term might be resurrected and used in the broad sense to include all of these relevant arthropods. Entomologists are students of *entoma*, and since entomology traditionally includes the study of mites and ticks, there should be no entomological violence in using the term broadly, especially since Aristotle used a broad definition of this same term. Thus insecticides and acaricides can be jointly considered as "entomocides" without violating our natural systems of taxonomy. In this sense "insect" would remain a term of genetic relationship, with "entomon" a term of ecological or disciplinary significance.

If all entoma were inhabitants of petri dishes, attention could be restricted essentially to the influence of various chemicals on internal physiological events. Since this is not the case, problems of behavior, of accessibility, and of differing environmental situations constantly intrude themselves into considerations of chemical control. The situation is further complicated when domestic animals are involved, since the host is also subject to disturbance by pesticidal chemicals. When the vertebrate host contributes to the public food supply, problems of residues and public health are also involved.

The entomon pests of livestock are of many types. Some are resident ectoparasites or endoparasites for all or part of their lives. Some are occasional or perennial visitors of their hosts. Lice, fleas, keds, and ticks are examples of ectoparasites that spend all their lives or long periods of their lives close to the skin of their hosts.

Another large grouping includes entoma that are resident obligate endoparasites or lesion inhabitors. Cattle grubs, horse bot flies, and sheep bot flies penetrate well within the bodies of their hosts. The various types of mites and the screw-worm and other blow flies inhabit various types of superficial and deep lesions. The relationships of many of these lesion inhabitors and the microorganisms that feed in the same wounds could be much better understood than they are at present.

A class apart from the resident entoma are the various insect visitors. Horn flies, while maintaining close relationships with their hosts, still retain a considerable degree of independence. A still higher degree of independence is seen among insects such as horse flies, stable flies, black flies, and mosquitoes.

All of these relationships of entoma with their hosts influence the nature of the entomocides used. Thus, even though a particular chemical may be highly toxic once introduced into the body of a particular arthropod, it may prove to be of minor effect if sufficient quantities cannot be conveniently introduced under practical conditions of treatment.

Another ecological consideration in the chemical control of livestock pests is the breeding site. Local breeders, whether on the host or in its immediate vicinity, are much more easily subject to control than distant breeders, such as mosquitoes or black flies, whose breeding grounds may be miles away from the animals to be protected. Local breeders, such as horn flies, are subject to control by treatment of the host, but stable flies are attacked with difficulty by treating the host alone, and mosquito populations remain virtually unaffected by host treatments. Breeding site control, except in the immediate vicinity of farm structures, is usually not within the practical reach of the agriculturist. The community approach available to many public health workers is usually prohibitively expensive for the agriculturist in sparsely inhabited regions.

Entoma vary in their susceptibility to entomocides. This variation is important on an individual as well as on a species basis. Some species, such as horn flies, have remained highly susceptible to a great many insecticides despite a high selection pressure. Stable flies, on the other hand, have always been difficult to reach with the insecticides at hand. Emergent resistance has come to plague livestock entomology no less than other phases of the profession.

A final word in this introduction should be devoted to the economic importance of entoma affecting livestock. This is an area that has been particularly weak academically. In the case of an insect such as the screw-worm, the economic importance is obvious, since disaster can follow an uncontrolled infestation. The relationship of blood-sucking lice and anemic conditions in mammals is reasonably well known (146,153), as is that between biting flies and milk production (21). In contrast, the effects of keds on sheep are much less obvious. Bosman *et al.* (12) showed that while keds did not affect weight gain of merino sheep, they adversely affected wool quality when feeding conditions were poor. Muma *et al.* (143) found no significant differences in weight gains following control of keds by various procedures. Whiting *et al.* (210) also found no relationship between weight gain or wool production and degree of infestation of keds on feeder lambs.

Several investigations have failed to disclose a clear relationship between cattle grubs and physiological responses of the hosts, such as weight gains, though many conflicting reports in the literature exist (94). The economic relationships between the cost of treatment and the return to the producer need much more precise evaluation, on a strictly academic basis, than they have had until now. Industry and extension surveys have given much useful information, but with not nearly the exactitude necessary for a clear understanding of these problems. Although the economic reasons for controlling the pests involved are frequently the compelling ones, the stockman is also concerned with the comfort of his animals, and even the esthetic value of a complete hair coat or of an entoma-free animal is sometimes sufficient compensation for many control programs.

II. Chemical Control Procedures

Chemicals used to control arthropod pests of domesticated mammals produce their effects in a variety of ways. The most obvious effects are those which so seriously interfere with normal physiological processes as to cause the death of the arthropod. Unfortunately, the physiological processes of arthropods are too similar to those of mammals to provide absolute distinctions between serious interference in the one and assured noninterference in the other. True, empirical results have disclosed innumerable situations where serious interferences in the arthropod are accompanied by relative safety to the mammal, but this demonstration of relative safety is a negative quantity and may simply reflect our ignorance of events outside the range of our investigations. There is no reason to believe that the cigarette–lung cancer relationship did not exist 50 years ago. The negative information available at that time implied a degree of safety which now appears unwarranted.

The problem of differential toxicity of chemicals to arthropods and to mammals is probably the most serious one associated with the use of these toxicants. While there are still many insects of economic importance for which effective control is lacking, an overwhelming number of arthropods can now be practically and readily controlled. In each case, however, the degree of practicality is limited, by the known or potential effects of these chemicals on the livestock being protected and—of far greater significance—on the human consumer.

The chronic effects of physiologically active chemicals have been of prime concern to the academic scientist no less than to those in commercial or regulatory fields. Unfortunately, too many of the decisions necessary in this vital field are based on empirical demonstrations rather than on sound understanding of the underlying physiological events. Until our understanding of the physiology of toxicants is at a considerably higher level than at present, dependence will continue to be placed on empirical demonstrations, and decisions concerning relative safety will rest on the negative approach of "toxicity not demonstrated." Thus, there will continue to be a degree of uncertainty concerning the actual safety of the materials in use, or of their metabolites. While some doubt about the absolute safety of certain materials now in use cannot be denied, it should be emphasized that regulatory agencies have properly adopted an attitude of great conservatism, and that such practical compromises as have now been made combine the highest degree of safety for the public with the practical demands for effective pesticides. If the regulatory agencies in the United States are to be criticized at all, it is in the direction of excessive attention to the public relations aspects of their functions. Thus it is this author's belief that the negative attitude toward DDT usage is predicated as much on unfavorable publicity for this chemical in the popular press as on actual scientific demonstrations, with the result that this material is more stringently regulated than other comparable materials.

Quite plainly, risk is one of the biological facts of life. Civilization, in reducing some of the dangers of primitive life, necessarily introduces certain other dangers. Adequate food for humanity is a boon that far outweighs the potential health hazards of pesticides. However, any reduction of this hazard would be most desirable.

Although toxicity is the major purpose of the use of chemicals, the interferences of behavior caused by repellents and attractants are also of considerable importance. The actual effects of these behavior modifiers are considerably more complex than is implied in the words repellent and attractant. Thus, locomotor responses may be increased, decreased, or modified to turning reactions, and feeding, mating, or oviposition behavior may be affected (6,139). More likely than not, chemical agents have multiple effects, toxicity-wise as well as in the modification of behavior (171).

A. LOCALIZED CONTROL

Localized chemical control procedures consist of treatment of the premises and treatment of the hosts. In the first category, space sprays have long been and still are highly practical for controlling such insects as flies and mosquitoes. Fixed space spray installations in dairy barns permit easy periodic treatment with a minimum of trouble and at reasonable cost. The development, over the past 15 years, of DDT and the host of other residual materials gave hope of an ideal long-term premise insect control. Although these materials are still extremely useful, the emergence of resistant insects and the problems associated with contaminating residues have greatly limited their value. Treatment of manure and other organic wastes for fly control is occasionally practiced. While mass spraying of premises was popular in the late forties, in the fifties emphasis shifted to localized or "spot" spraying, to liquid or dry "baits" of various types (54,74,92,96,102,138,150,200), and to insecticide-treated cords suspended in various ways from the ceiling or in front of windows (8, 103,150,185).

Direct treatment of the host is the method used to control the greatest number of entomon parasites, highly effective entomocides of broad "spectrum" being available for most mammal-infesting ectoparasites. When chemicals are applied to livestock, the question of method of application must be considered in addition to the problems of effectiveness, mammalian toxicity, and potential contamination of the public food supply. Often the choice of method is determined by the economics of control operations, since several effective. formulations and methods of application, as well as formulation, are available for most chemicals. Where a pest is of sufficient economic importance, a limited number of formulations and difficult methods of application can continue to be employed. For example, the practices involved in screw-worm control in some southern states of routinely examining stock, capturing individual animals, and daubing a smear formulation on their wounds with a paintbrush are certainly laborious and can be justified only where refraining from these practices can result in disaster.

The choice of a method of application is also influenced by the size and type of farm. Numbers of animals, management practices in their routine handling, and availability of equipment greatly affect the nature of the application procedure adopted. Spraying equipment enjoys wide popularity, largely because of its portability and ease of handling. Where the operation is large enough, the sprayer that develops in excess of 400 p.s.i. at the pump is common, while smaller operators frequently treat their animals with smaller units of considerably less efficiency and versatility. It should be noted that pump pressure as a rule of thumb for characterization of a machine can be greatly abused or misunderstood (132); actually it has validity only as pump pressure is correlated with output, nozzle type and versatility, and agitation mechanism.

Where complete coverage and thorough wetting of the skin are desired, the dipping of livestock is probably the most efficient procedure. While fairly convenient for sheep, dipping is quite a troublesome operation for cattle and requires relatively expensive installations that are frequently less adaptable for use on individual ranches than is spray equipment. Many insecticides are available in special formulations for use in dipping vats, formulations designed for ease of suspensibility or avoidance of "creaming" or scum formation. Dingle (41) has discussed the problems associated with the use of DDT and BHC suspensions in cattle dipping baths in Australia, and Mahoney (134) has provided directions for the operation of DDT dipping baths.

Power dusting of freshly sheared sheep for the control of keds and blowflies is frequently practiced. Pfadt and DeFoliart (155,156) demonstrated the high effectiveness of this method and the minimal residues obtained therefrom, and in 1956 DeFoliart (34) reported dusting to be as effective as spraying for horn fly control on beef cattle. There are several obvious advantages to dusting, principally that the outlay for equipment is lower than for sprays and water is not needed.

Space sprays in barns are also used to control flies resting on cattle as well as in the rest of the area. Where such treatments are included in daily routines, they can be very successful in mitigating the effects of horn flies and of stable flies (60,204).

The expense, in terms of labor, of many of the application methods mentioned above has led to the development of a variety of self-treatment devices for cattle. Low pressure, low volume spraying systems, activated by individual cattle were pioneered by Bruce (16,18), who devised a treadle-operated system. The apparatus is set in a passageway through which the animals must pass, and their weight on the treadle activates the sprayer mechanism. Treadle sprayers have been very effective in controlling horse flies and other biting flies of cattle, but mechanical difficulties have been encountered with the treadles. This has led to the development of lever-operated systems

(115) and systems activated by a photoelectric cell (27) in which the sprayer itself is operated by supplementary power. The individually activated spraying systems are most appropriate for dairy animals or in other situations where equipment maintenance is not too difficult.

Rubbing devices, such as the cable-type backrubber initially described by Rogoff and Moxon (176), have been particularly successful for the control of horn flies under range and pasture conditions (see also 27,61,69,76,164). Hoffman (84,85) found that cable-type backrubbers of several designs gave practical control of lice on cattle. Mahoney (135) found these devices practical for buffalo fly control in Australia. The low initial expense and operating cost, coupled with tissue residues considerably lower than obtained after routine spraying (27,176), are among the major advantages of this system. The major limitation of the backrubber is that under some circumstances cattle may ignore the device. Careful placement of the units in locations where cattle loaf seems to be more important than the distraction of trees or other competitive rubbing places. The many variations in design of these devices seem to be of only minor significance, if any. Several of the more simple commercial designs have an edge in terms of convenience, while the more expensive and elaborate instruments seem to lack any advantage beyond sales appeal. Residue studies for reservoir-type units have not been seen by this reviewer, and there may actually be disadvantages, in terms of residues, to their use.

The recent development of systemic agents for controlling animal ecto- and endoparasites has added a new dimension to livestock entomon control. Where the previously discussed dissemination methods involve the host only externally, with the systemics the toxic material reaches the parasite via the physiological system of the host. Now to the spray machine and other familiar tools of the entomologist have been added the balling gun to introduce oral boluses or oblets, and the hypodermic needle for parenteral injections. Feed and salt have also been used for introduction of systemic chemicals into mammals (109,109b,174).

The history of the development of systemic agents began over three decades ago. In 1928 Parman *et al.* (151) unsuccessfully attempted to use internal medication for the control of poultry ectoparasites. Significant, though immediately impractical, demonstrations of systemic activity were given in 1944 by Lindquist *et al.* (124), in 1946 by DeMeillon (37), and in the following decade by many others (2,3,47,57,107,125,130,182,190).

For a short period following the initial successes of Worden in 1953 (212) and Schwartz *et al.* in 1954 (188), there was considerable excitement over the possibility of using phenothiazine to control cattle grubs. This excitement, which spread to the farm papers, was short-lived, however, as later evidence indicated no promise for this procedure (109b,166,169,173).

The development of ronnel for the control of cattle grubs (31,121) was the first major, practical success for a systemically active chemical. The last five years have seen this area of research expanded to such proportions that a complete review of publications would be beyond the scope of this article. The development of ronnel, Co-Ral,* dimethoate, Dowco 109,** and currently of Ruelene** has been widely reported at technical meetings and in the literature (1,23,25,77, 80,94,95,109b,129,133,152,165,172,174a,174b,179,203). Radeleff and Woodard (160) discuss some of the toxicological problems in the use of systemic insecticides for livestock.

B. POPULATION CONTROL

The control of entire pest populations is a problem of an entirely different order of magnitude than the localized control operations on a relatively small number of host mammals within the boundaries of a single farm or ranch. Control of lice, keds, or mange is entirely practical within a herd, but the control of cattle grubs or screw-worms is only of short-term value if not practiced over large geographical areas.

The great tick control campaigns of the past in North America are now classical. These campaigns were conducted in situations of great medical and economic urgency. Their success has served as models for many similar attempts. More recent examples are given by Hall (71) and Lancaster (114).

The term "eradication" fell into bad repute a generation ago in the wake of an overoptimism as to the capabilities of our insect control techniques. There was a resurgence of interest with the introduction of DDT, and fresh discouragement with the widespread development of insecticide resistance. Then the systematic and widely successful malaria eradication programs revived hope for application of population control to the entoma that affect livestock. Now the release of vast numbers of sterile but vigorous male flies is proving effective in eliminating screw-worms. Bushland and Hopkins (26) have discussed many of the problems involved in large-scale rearing and sterilization of screw-worm larvae. A more elaborate account of the eradication program as it was going in 1958 was given by the Florida Livestock Board and the United States Department of Agriculture (51), and the entire situation has been reviewed by Bushland (25a) in Volume III of this series. Hocking and Richards (83) demonstrated effective area control of black flies, with successful results 2–6 miles from the periphery of a circular area 16 miles in diameter. Lea and Dalmat (119) obtained promising results in a pilot study on area larval control of black flies in Guatemala. In experiments on range herd horn fly control, Denning and Pfadt (39) noted that an early application of insecticide, when few flies were present, did not lessen the total number of spray applications required for the season. Smith and Gates (192) describe a 720 square mile area where DDT was used against horn flies with excellent results, but without achieving eradication. Community fly and mosquito control campaigns are, of course, further examples of population control.

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* Registered trademark Chemagro Corp., Kansas City, Mo.

** Registered trademark The Dow Chemical Co., Midland, Mich.

Another example of population control is Norris' "strategic dipping" (148), used in Australia for the control of the cattle tick, *Boophilus microplus* (Canestrini). The periodic dipping of cattle is timed to provide control just short of eradication, the purpose being to reduce tick populations, but still maintain the immunity of the cattle to redwater fever.

It is probably wise to maintain a conservative attitude toward absolute eradication of species. Not only would there be many uncontrollable variables in any such campaign, but they would also be to some extent unpredictable. It is plain, however, that whether the procedure is biological or chemical, control of large populations is coming more and more into the realm of the practical.

In recent years there have been a number of large-scale programs for control of populations of cattle grubs (7,126). Many relatively successful campaigns of this sort were carried on in Europe, even by the laborious technique of squeezing the grubs from the backs of cattle by hand. Denmark instituted a country-wide project that was limited only because no similar program was adopted by her neighbors in Germany. In Switzerland the control of *Hypoderma* has been officially organized since 1921 and obligatory since 1944 (56).

At present considerable interest is developing in the possibility of using the recently developed systemic agents in connection with renewed attempts to control cattle grub populations. Destroying the grubs in the backs of cattle is hardly more than revenge unless reinfestation the following year is also prevented. The flight range of the adult is sufficiently great that control of large populations is necessary before any long-term effects can be seen. The coming of the highly effective and easy to use systemics permits coupling short-term advantages with the over-all pattern of population control.

III. Effectiveness of Chemical Control Procedures

For all practical purposes, at present, the control of entoma on livestock depends on chemical agents. Mechanical procedures such as hand picking, traps, and sticky paper have value, but on so small a scale as barely to enter the picture in North America. Insect diseases are not used against the pests under discussion, nor are introduced parasites or predators of significance, though top-feeding minnows have been used for mosquito control in appropriate situations. The only current biological control of note is the saturation of wild populations with sterile male screw-worm adults. Probably the most important nonchemical procedure is the reduction of breeding sites by sanitation or other cultural practices. Sanitation practices are of prime importance in fly control campaigns, and certainly drainage or filling operations are critically important in many mosquito control situations. Under agrarian conditions, however, sanitation practices can rarely be sufficiently complete to replace chemical agents for practical control operations. This is not to minimize the importance of sanitation, but rather to stress that of chemical control.

The successes of chemical control procedures during the past 15 years are most impressive. A long list of insects formerly difficult to control are now managed with relative facility. Some that were not too difficult to control are now controllable more easily and more cheaply. Despite these successes, the pattern is not quite as simple as was anticipated in 1946. Though large numbers of insects have shown susceptibility, insecticide resistance developed and continues to increase. Among the many excellent reviews of specific chemical control procedures and their effectiveness may be mentioned those by Harbour (75), Fuller (52), Lindquist (122), Lindquist and Knipling (123), and Fay and Kilpatrick (49). The resistance problem has been most competently reviewed by Brown (13).

In spite of the manifold types of resistance that have developed in house fly populations, the chemical campaign against this insect can certainly be regarded as highly successful in comparison with previously available procedures. Although discouragement set in following the peak use of the chlorinated organic entomocides, this has now been largely dissipated through the success of the organophosphates. Resistance is also developing to these newer insecticides (13) but much more slowly. Furthermore, the existence of other chemical groups, such as the carbamates (193a), which are also highly effective, provides considerable hope for the immediate future at least.

Chemicals have also been very successful in horn fly control. Here not only are the earlier chlorinated organics still effective, but a whole new set of chemical groupings has come onto the scene. Horn flies appear to be susceptible to most insecticides.

The many successful procedures should not obscure procedures that are of just passable efficacy or problems that are still essentially unsolved. One insect that has a major effect on many classes of livestock is the stable fly. Although its numbers can be partially controlled through sanitary practices, this pest remains a regular visitor even when sanitation is relatively good. Available chemical procedures can mitigate the problem, but under circumstances that are barely practical. Where relatively frequent treatment is economically justifiable, these flies can be killed or repelled, but where only infrequent treatment is practical, as for range cattle, the results leave much to be desired.

Another problem that is far from solved is the control of mosquitoes on cattle (209). Part of the problem here, as it is also but to a lesser extent with stable flies, is that even where a chemical produces high mortality among attacking mosquitoes, this action is seldom rapid enough to prevent attack. It becomes, therefore, a revenge type treatment that punishes the attacker but which does not prevent further attack. In this insect control problem, where enormous numbers of potential feeding individuals are frequently present, success can be achieved by blocking the feeding response or inducing the mosquito to depart before feeding, but not by killing the insect after it has been fed. Unfortunately, the behavior-modifying chemicals are not active for sufficiently long periods to be practical where frequent treatment is out of the question.

IV. Materials in Use or Development

A great many chemicals are in use and in various stages of development for the control of entoma on livestock. It is obviously not possible to present anything like a complete list of materials in use, and even harder to do justice to a survey of materials under development. Plainly, only the more obvious materials can come under scrutiny, and even so it is possible that important materials will be overlooked while less significant materials might be included.

A. UTILITY

The more common chemicals in recent or current commercial or experimental use, together with the entoma against which a significant degree of utility has been claimed in the literature for each chemical, are listed on pages 166–173. The citations are typical rather than complete. It should not be assumed that any recommendation is implied herein for each use mentioned. Some uses have not yet gained general acceptance, some may be impractical, some may be already obsolete. Formal current recommendations should be obtained from appropriate official governmental agencies. In the main, however, this listing should provide a view of the chemicals and the entoma against which they have been applied during the past decade.

B. PHYSIOLOGICAL EFFECTS ON MAMMALS

Most of the entomocides in actual use on, or in the vicinity of, domesticated animals normally appear to have slight if any adverse effects on the livestock. Obviously this is a necessary qualification for an economic pesticide. In most situations severe overdosing can be tolerated (183). Under physiological stress, however, materials normally quite safe may become dangerous and severe losses may follow their use. Radeleff and Bushland (159) describe severe poisoning of emaciated sheep following routine dipping in BHC.

Common Entomocides in Recent or Current Commercial or Experimental Use*

Entomocide	Entoma	Ref.
Aldrin	Cattle grubs	131
	Horse flies (larvae)	90
	House flies (larvae)	70,184,197,207
	Human bot flies (larvae)	145
	Keds	155
	Screw-worms	131
	Sheep blow flies (larvae)	141,142,196
	Ticks (cattle)	182
Allethrin	Horn flies (adults)	67
	House flies (adults)	207
	Stable flies (adults)	67
Arsenic (As_2O_3)	Sheep blow flies (larvae)	141
	Ticks (cattle)	206*
BHC (see also Lindane)	Black flies (larvae)	154
	Blow flies (larvae)	68
	Cattle grubs (preinfestation)	66,110
	Horn flies (adult)	39
	House flies (adults)	207
	House flies (larvae)	207
	Human bot flies (larvae)	111,145
	Keds	40,48,97,110,189,191
	Lice (cattle)	113
	Lice (swine)	98
	Lice (sheep)	40,97,191
	Mites (cattle)	186
	Mites (dogs)	144
	Mites (goats)	186
	Mites (sheep)	110,167,202
	Mites (swine)	30,81,98,170
	Mosquitoes (adults)	207
	Mosquitoes (larvae)	207
	Sheep blow flies (larvae)	93,142,196
	Ticks (area treatment)	127,207
	Ticks (cattle)	28,29,147,149,182
	Ticks (ear ticks)	100
Chlordane	Black flies (larvae)	154
	Fleas (adults)	207
	Horn flies (adults)	39,46,76
	Horse flies (larvae)	90
	House flies (adults)	206*,207
	House flies (larvae)	70,184,206*,207

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* Ref. 206 indicates uses recommended in 1960 by the U. S. Department of Agriculture. For qualifications, see this reference or its annual replacement.

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
	Keds	48,86,189
	Lice (cattle)	35,85,106,113
	Lice (horses)	10
	Lice (swine)	98
	Mites (dogs)	144
	Mites (swine)	98,170,195
	Mosquitoes (adults)	207
	Mosquitoes (larvae)	207
	Stable flies (adults)	206*
	Ticks (area treatment)	64,114,127,206*,207
	Ticks (cattle)	24,106
Chlorthion[a] (Bayer 22/190)	Blow flies (adults)	96
	Cattle grubs	130,178
	House flies (adults)	55,73,74,92,104
	Lesser house flies (adults)	74
	Lice (cattle)	193
	Lice (goats)	193
Co-Ral[a] (Bayer 21/199)	Cattle grubs	23,25,36,43,77,174b, 178,203,205,206*
	Horn flies (adults)	22,76,204,206*
	House flies (adults)	55
	House flies (larvae)	197
	Keds	155,177,206*
	Lice (cattle)	36,193,206*
	Lice (goats)	193
	Lice (sheep)	206*
	Lice (swine)	206*
	Screw-worms	206*
	Sheep blow flies (larvae)	206*
	Ticks (cattle)	43a,206*
	Ticks (sheep)	206*
Cyclethrin	Horn flies (adults)	67
	Stable flies (adults)	67
DDT	Black flies (larvae)	4,59,83,119
	Blow flies (larvae)	68
	Buffalo flies (adults)	135
	Cattle grubs	108
	Cattle grubs (preinfestation	11
	Fleas (adults)	207
	Horn flies (adults)	32,33,39,46,128,146, 176,192,206*

(*continued*)

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
	Horse flies (area treatment)	14
	Horse flies (larvae)	58,90
	House flies (adults)	8,55,101,105,206*, 207
	House flies (larvae)	207
	Human bot flies (larvae)	111,145
	Keds	86,143,155,177,187, 189,191,206*
	Lice (cattle)	35,84,106,193
	Lice (goats)	193
	Lice (horses)	10
	Lice (sheep)	191,206*
	Lice (swine)	99,206*
	Mites (cattle)	42
	Mites (horses)	42
	Mites (sheep)	42
	Mosquitoes (adults)	207
	Mosquitoes (larvae)	88,207
	Sheep blow flies (larvae)	44,93
	Stable flies (adults)	206*
	Ticks (area treatment)	64,127,206*,207
	Ticks (cattle)	5,9,29,137,147,148
DDVP	House flies (adults)	55,102,207
	Lesser house flies (adults)	150
	Mosquitoes (adults)	207
Diazinon[b] (Geigy 24480)	Cattle grubs	130
	Fleas (adults)	207
	Horn flies (adults)	60,203
	House flies (adults)	33,54,55,72–74,92, 103–105,118,185, 206*,207,211
	House flies (larvae)	87,184,197,207
	Keds	155
	Lesser house flies (adults)	74,150
	Lice (cattle)	193
	Lice (goats)	193
	Mosquitoes (adults)	207
	Sheep blow flies (larvae)	50,141,196
	Stable flies (adults)	206*
	Ticks (cattle)	120
Dicapthon (American Cyanamid 4124)	House flies (adults)	55,73,74,92,104
	Keds	155,177

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
	Lesser house flies (adults)	74
	Lice (cattle)	193
	Ticks (cattle)	43a
Dieldrin	Cattle grubs	131, 181
	Horn flies (adults)	32, 33
	Horse flies (area treatment)	14
	Horse flies (larvae)	90
	House flies (adults)	207
	House flies (larvae)	70, 197, 207
	Keds	155–157
	Lesser house flies (adults)	207
	Lice (cattle)	35
	Mosquitoes (adults)	207
	Mosquitoes (larvae)	207
	Screw-worms	45,131
	Sheep blow flies (larvae)	91, 141, 142, 196
	Ticks (area treatment)	64, 114, 206*, 207
	Ticks (cattle)	182
Dimethoate (American Cyanamid 12880)	Cattle grubs	36, 80, 174b, 203
	Lice (cattle)	36
Dipterex[a] (Bayer L 13/59, Dylox[a])	Bed bugs	2
	Blow flies (adults)	96
	Cattle grubs	130, 178
	Fleas (adults)	207
	House flies (adults)	17, 33, 54, 55, 73, 74, 92, 96, 104, 105, 118, 206*, 207
	House flies (larvae)	87, 197, 207
	Keds	177
	Lesser house flies (adults)	74
	Lice (cattle)	193
	Mosquitoes (larvae)	207
	Stable flies (adults)	206*
	Ticks (rabbits)	2
	Ticks (cattle)	43a
Dowco 109[c] (Narlene)	Cattle grubs	36, 174b, 180, 203
	Human bot flies (larvae)	133
	Keds	177
	Lice (cattle)	36
Endrin	House flies (larvae)	184, 197
	Lice (goats)	193
	Sheep blow flies (larvae)	142

(*continued*)

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
EPN	House flies (adult)	55, 105
	House flies (larvae)	197
	Lice (cattle)	193
	Lice (goats)	193
	Mosquitoes (larvae)	88
Heptachlor	Blow flies (adults)	175
	Horn flies (adults)	32
	Horse flies (larvae)	90
	House flies (larvae)	70, 184
	Keds	155
	Lice (cattle)	35
	Mites (dogs)	144
	Mosquitoes (larvae)	207
	Ticks (area treatment)	114
Lethane[d]	Horn flies (adults)	206*
	Horse flies (adults)	206*
	Mosquitoes (adults)	206*
	Stable flies (adults)	206*
Lindane (see also BHC)	Cattle grubs	131
	Fleas (adults)	207
	Horn flies (adults)	63, 112, 203
	Horse flies (adults)	63
	Horse flies (area treatment)	14
	House flies (adults)	74, 112, 200, 206*, 207
	House flies (larvae)	184
	Human bot flies (larvae)	198
	Keds	155, 206*
	Lesser house flies (adults)	74
	Lice (cattle)	35, 113, 206*
	Lice (sheep)	206*
	Lice (swine)	98, 206*
	Mites (cattle)	62, 158
	Mites (swine)	98, 170, 199
	Mosquitoes (adults)	207
	Screw-worms	15, 45, 131, 206*
	Sheep blow flies (larvae)	206*
	Stable flies (adults)	206*
	Ticks (area treatment)	206*
	Ticks (cattle)	43a, 106, 206*
	Ticks (ear ticks)	206*
	Ticks (sheep)	206*

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
Malathion	Fleas (adults)	207
	Horn flies (adults)	206*
	House flies (adults)	34,54,55,73,74,92, 96,101,104,118, 138,206*
	House flies (larvae)	184,197
	Keds	155,177,206*
	Lesser house flies (adults)	74,150,207
	Lice (cattle)	35,193,206*
	Lice (goats)	193
	Lice (sheep)	206*
	Lice (swine)	206*
	Mites (swine)	163
	Mosquitoes (adults)	207
	Mosquitoes (larvae)	207
	Stable flies (adults)	206*
	Ticks (cattle)	43a,120,206*
	Ticks (sheep)	206*
Methoxychlor	Horn flies (adults)	27,39,46,76,112,128, 146,206*
	House flies (adults)	34,74,112,206*,207
	Keds	86,155
	Lesser house flies (adults)	74
	Lice (cattle)	85,106,206*
	Lice (sheep)	206*
	Lice (swine)	206*
	Stable flies (adults)	206*
Parathion	House flies (adults)	103,105,185
	House flies (larvae)	184,197
	Lesser house flies (adults)	150
	Lice (cattle)	193
	Screw-worms	45
	Mosquitoes (larvae)	207
Pirazinon[b] (Geigy 24622)	House flies (adults)	74,92
	Lesser house flies (adults)	74
	Lice (cattle)	193
Pyrethrum (commonly with synergist)	Fleas (adults)	207
	Horn flies (adults)	39,63,67,112,206*
	Horse flies (adults)	19,63,89,206*
	House flies (adults)	112,206*,207
	Keds	187,206*
	Lice (cattle)	194,206*
	Lice (swine)	140

(*continued*)

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
	Mosquitoes (adults)	206*
	Stable flies (adults)	20,67,82,206*
	Ticks (cattle)	5,206*
Ronnel (Dow ET-57, Dow ET-14, Trolene,[c] Korlan[c])	Cattle grubs	1,25,36,77,94,95, 129,165,174b,179, 203,205,206*,208
	Horn flies (adults)	204
	House flies (adults)	55,73,105,206*,207
	House flies (larvae)	87
	Keds	155,177
	Lice (cattle)	36
	Lice (sheep)	206*
	Lice (swine)	206*
	Mosquitoes (adults)	207
	Screw-worms	206*
	Sheep blow flies (larvae)	206*
	Sheep bot flies (larvae)	152
	Stable flies (adults)	206*
	Ticks (cattle)	43a,206*
Ruelene[c]	Cattle grubs	174a,109b
	Ticks (cattle)	43a,
Rotenone	Cattle grubs	53,168,206*
	Fleas (adults)	207
	Horse flies (adults)	89
	Keds	38,40,65,143,155, 187,206*
	Lice (cattle)	113,136,206*
	Lice (sheep)	40,206*
	Ticks (cattle)	5,206*
Sevin[e] (Union Carbide)	Horn flies (adults)	78
	Ticks (cattle)	43a,117
Sodium arsenite	House flies (larvae)	184
	Lice (sheep)	65
Strobane[f] (B. F. Goodrich, 3961)	Lice (cattle)	193
	Lice (goats)	193
	Ticks (cattle)	24
Sulfur	Keds	143,187
	Lice (cattle)	136
	Mites (cattle)	62
TDE	Horn flies (adult)	39,46,128
	House flies (larvae)	184
	Lice (cattle)	106
TEPP	House flies (adults)	200

Common Entomocides (*continued*)

Entomocide	Entoma	Ref.
Thanite[g] (Hercules Powder)	Horn flies (adults)	206*
	Horse flies (adults)	206*
	Mosquitoes (adults)	206*
	Stable flies (adults)	206*
Thiourea	House flies (larvae)	184,207
Toluene	Horse bot flies (larvae)	201
Toxaphene	Horn flies (adults)	46,76,112,128
	House flies (adults)	112
	Human bot flies (larvae)	111,145
	Keds	48,86,155,156,189, 206*
	Lice (cattle)	85,106,206*
	Lice (sheep)	206*
	Lice (swine)	98
	Mites (swine)	98
	Stable flies (adults)	206*
	Ticks (area treatment)	64,127,206*,207
	Ticks (cattle)	24,43a,106,116,137, 206*
	Ticks (sheep)	206*

[a] Registered trademark Chemagro Corp., Kansas City, Mo.
[b] Registered trademark Geigy Chemical Corp., Ardsley, N. Y.
[c] Registered trademark The Dow Chemical Co., Midland, Mich.
[d] Registered trademark Rohm & Haas Co., Philadelphia, Pa.
[e] Registered trademark Union Carbide Corporation, New York, N. Y.
[f] Registered trademark B. F. Goodrich Chemical Co., Cleveland, Ohio.
[g] Registered trademark Hercules Powder Co., Wilmington, Del.

Where the animals are not being treated directly, or where the pest is as easily reached as the host (as in treatments for horn flies) difficulty is rarely seen. The use of chemicals as systemic agents, however, requires a relatively high degree of exposure of the host. In ronnel's first year of commercial use, despite previous indications of safety, hundreds of cattle were affected, with deaths reported in many parts of the United States. The proportion of animals injured compared to the total number treated was low, but was sufficiently high to have a sobering influence on the development of systemics. When individual handling is required, as in balling-gun operations, occasional serious mechanical injury occurs.

A good general review to 1955 of the acute toxicity of various chlorinated hydrocarbon and organic phosphorus insecticides to livestock was given by Radeleff *et al.* (162). A summary of pesticide residues in meat and milk was presented by Claborn *et al.* (27a).

The recent popularity of the organophosphate entomocides has led to considerable interest in erythrocyte cholinesterase levels as indicators of degree of exposure. While the degree of cholinesterase inactivation varies greatly, there is sufficient reliability relative to those compounds that are strong inhibitors to provide a useful, if not completely valid, tool. Hermenze and Goodwin (79) and Radeleff and Woodard (160a) provide data on the normal cholinesterase activity in the erythrocytes of cattle. The lowering of erythrocyte cholinesterase levels, however, is not necessarily an unfavorable reaction. Such depression may or may not be correlated with depletion of this enzyme in critical tissues. Depression of cholinesterase levels in the red blood cells, when accomplished at a sufficiently slow rate, has not been associated with deleterious physiological events (161).

V. Outlook

Outstanding advances in the chemical control of insect pests of domesticated animals have been registered in the past two decades. As has been shown in this review, a considerable variety of materials have been tested and made commercially available. It would be reasonable to expect that the next two decades will provide materials that will make obsolete many entomocides now in common use. While no great dangers to public health are inherent in current entomocidal practices, the necessity of reducing such risks to the lowest possible levels may result in great emphasis on behavior-modifying chemicals. Exciting research possibilities exist for the development of chemicals and devices that would affect the behavior mechanisms of noxious insects in such a manner as to make inanimate objects, traps, or toxic surfaces competitive with livestock for the attention of these entoma.

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RESIDUE DETERMINATION BY CHOLINESTERASE INHIBITION ANALYSIS

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I. Introduction

It is now generally recognized that insecticides should not be used to treat edible crops until experience has shown that, at the recommended application rates, any insecticide residue remaining at harvesting is insufficient to endanger the health of the consumer. Many countries now follow the precedent set by the United States and demand that manufacturers wishing to market insecticides must first reveal details of residues which may be expected on crops treated in the recommended manner, as well as details of the analytical methods by which these results have been obtained. In addition, public analysts may be required to sample and analyze crops of known or unknown origin to ensure that no insecticide is present in excess of an accepted tolerance figure. Much attention has, therefore, been directed to the elaboration of analytical methods of suitable sensitivity and specificity to measure the very small quantities which careful experiments on animals or on man have shown to be permissible as residues. The organic phosphate and carbamate insecticides owe their toxic action to their ability to inhibit the enzyme cholinesterase in insects and in mammals, and the possibility of effecting this inhibition *in vitro* with some members of the group has led to the introduction of analytical methods which make use of the same enzyme-inhibitor reaction which is responsible for the biological action. In this chapter the theory and practice of residue determination by cholinesterase inhibition methods will be discussed, and the suitability of these methods for the routine control of residues will be considered. Apart from a description of a few techniques which have not hitherto been published, no attempt will be made to give a working description of procedures, for which the original publications should be consulted.

II. Measurement of Cholinesterase Activity

A. KINETICS OF CHOLINESTERASE ACTION

The natural substrate of the enzyme cholinesterase is acetylcholine, which is hydrolyzed by the enzyme to produce choline and acetic acid. It is customary to represent this reaction in the following manner:

$$E_f + S \underset{K'_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K''_2} E_f + P \tag{1}$$

Equation 1 shows that the free enzyme, E_f, forms with the substrate, S, a reversible complex, ES, which can also break down to yield the free enzyme and the hydrolysis products, P. The activity of the enzyme is measured by the rate of breakdown of the substrate $(-dS/dt)$ and is proportional to the concentration of the complex, ES. The differential equation for the breakdown of the substrate is given by equation 2, where K_S is the Michaelis constant of the enzyme and is equal to $(K'_2 + K''_2)/K_1$, and $(-dS/dt)_{max}$ is the rate of hydrolysis when the enzyme is all in the form ES and is a measure of the enzyme activity.

$$-dS/dt = (-dS/dt)_{max}\,(K_S/S + 1)^{-1} \tag{2}$$

If the substrate concentration is large enough not to be significantly changed during the course of the reaction, dS/dt is a constant for a given value of S and may be used as a measure of enzyme activity. Under this condition equation 2 shows that the effect of variation of S on dS/dt will be slight when S is much greater than K_S, which is desirable when enzyme activity is to be measured by the rate of appearance of hydrolysis products. The Michaelis constant of cholinesterase varies considerably according to the origin of the enzyme, but is usually in the region of 10^{-3} to 10^{-4} mole/liter, and the substrate concentration should, therefore, not be less than $2 \times 10^{-3}M$. Very much higher concentrations are to be avoided, however, as a large excess of substrate may tend to exert an inhibiting action on the enzyme. In inhibition studies where measurements of enzyme activity are made on the enzyme before and after incubation of the enzyme with the inhibitor, the initial substrate concentration used in the two measurements should be identical, or an error may be introduced.

When the enzyme activity is measured by recording the disappearance of the substrate, it is not possible to use an excess of substrate and dS/dt is no longer constant. Integration of equation 2 gives equation 3, where S_0 and S are the initial and final substrate concentrations, and t is the period of hydrolysis.

$$K_S \log_e S/S_0 - S_0 + S = t(-dS/dt)_{\max} \qquad (3)$$

The enzyme activity is given by $(-dS/dt)_{\max}$, and equation 3 shows that this is not a simple function of the values of S, S_0, and t. Enzyme activity measurements by this method are most simply achieved by first preparing a standard curve relating known amounts of enzyme to the measured change in substrate concentration, ΔS, keeping S_0 and t constant (see Section II.C.3). It is desirable that the enzyme activity should not be so high, nor the hydrolysis period so long, that the substrate concentration falls much below 50% of its original value, for below this figure the departure from linearity of the graph relating ΔS to time becomes marked, with an attendant increase in curvature of the standard curve relating ΔS to enzyme activity.

B. SOURCE OF ENZYME

Naturally occurring cholinesterases fall into two groups which have received a variety of names but which are probably best referred to as Group I and Group II (3). Although the natural substrate for both groups is acetylcholine, it is possible to distinguish between them by their substrate specificity; Group I enzymes, which are found in red blood cells and the central nervous system, hydrolyze β-methylacetylcholine much more readily than butyrylcholine, while the reverse is true of the Group II enzymes, which are most readily obtained from blood plasma. This substrate specificity is not of importance in residue analysis, although a few methods for measuring cholinesterase activity have been described in which special substrates are used. Of greater importance is the difference between these two groups of enzymes based on their specificity toward certain inhibitors. The sensitivity of inhibition analysis is a function of the interaction between enzyme and inhibitor, and the enzyme selected should be of the type most readily reactive with the substance under investigation. The possibility of using selective inhibition to distinguish among organic phosphates has been suggested, and as an example reference has been made to the greater effect of malathion on rat red cell cholinesterase than on the plasma enzyme, while Diazinon* has a reverse effect (7). These two compounds are not very good examples because they are not good *in vitro* inhibitors and their action is probably due to impurities. In the excellent review of this subject by Augustinsson (3) no reference is made to any organic phosphate at present in commercial use as a pesticide; nevertheless, in the application of inhibition analysis to a new compound it would be desirable to study the action of the compound on different types of enzyme.

Outdated human plasma obtained from a blood bank is frequently used for residue analysis and contains mainly Group II enzyme. Horse serum can be purchased and also provides a good source of cholinesterase. These preparations retain their activity for several months if kept in a refrigerator and not allowed to freeze. Purified bovine erythrocyte cholinesterase (Group I) is available commercially in the United States and is sold in 20,000 unit vials. (The unit is defined in terms of the method used to measure enzyme activity; in the commercial preparation it is that quantity of enzyme which will liberate 1 cu. mm. of carbon dioxide per minute in a Ringer bicarbonate buffer equilibrated against 5% carbon dioxide in nitrogen at pH 7.4 at 37.5°C., with an initial acetylcholine concentration of 0.0092M.)

In the author's laboratory a stable concentrated preparation of

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* Registered trademark Geigy Chemical Corporation, Ardsley, N. Y.

Group I cholinesterase is made from horse red cells, which are obtainable from certain manufacturers of serums. The cells are washed three times with 0.9% saline and then hemolyzed by diluting to four times their original volume with distilled water. The product is adjusted to pH 6.3 and centrifuged, the supernatant liquor is discarded, and the deposit is washed twice with 0.9% saline containing 0.01*M* phosphate buffer, pH 6.3. The deposit is homogenized in 0.9% saline containing 0.01*M* phosphate buffer; 0.5% gelatin and 10 p.p.m. aureomycin are added; and, after adjustment of the pH to 8, the suspension is left for 1 hour and then centrifuged. The supernatant liquor is retained, and the deposit re-extracted in the same manner. The two extracts are bulked together, their total volume being about four-fifths of the original red cell volume. The pH is adjusted to 7.4, and the solution filtered through a cotton wool plug, filled into 5 ml. ampoules, and stored in a refrigerator at −30°C.

C. METHODS OF MEASURING CHOLINESTERASE ACTIVITY

1. *pH Change Method*

When cholinesterase acts on its substrate, acetylcholine, there is a fall in pH due to the liberation of acetic acid. A method of determining the cholinesterase activity of human red cells and plasma, based on the potentiometric measurement of pH change produced under standard conditions, was developed by Michel (21). Michel realized that the rate of change of pH for a given enzyme activity was dependent mainly on buffer strength of the solution, and that the presentation of the results would be simpler if the buffer composition produced a linear change of pH with time. He developed barbiturate–phosphate–chloride buffers for red cell and plasma solutions and described a procedure whereby the fall in pH could be corrected for the effect of pH on enzyme activity and on the nonenzymic hydrolysis of the substrate. This method is one of the most attractive for residue analysis because it requires a minimum of manipulation, and it has been widely used. The method was first introduced for this purpose by Giang and Hall (11) and was subsequently adapted and used by a number of other workers (15). It was claimed that the modification of the buffer introduced by Giang and Hall gave a better linear relation between pH and time, leading to a direct proportion between ΔpH and enzyme activity. Their published ΔpH-activity graph had, however, a marked curvature over the range pH 8–6, though other

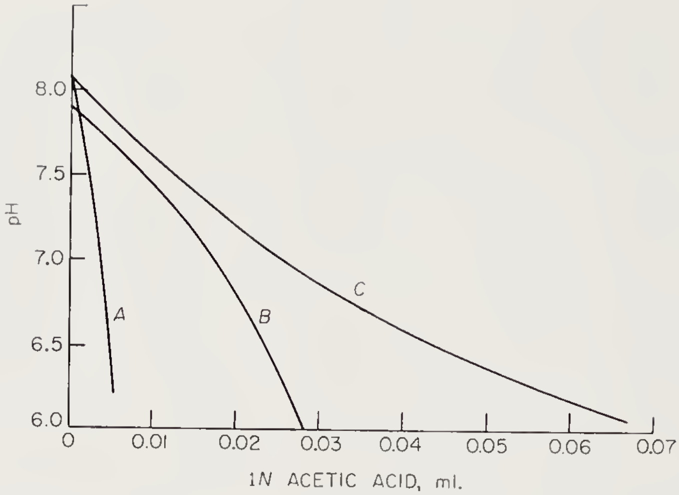


Fig. 1. Titration curves of buffer mixtures. Curve *A*, 1 ml. of Michel buffer; curve *B*, 1 ml. of Giang and Hall buffer; curve *C*, 1 ml. of Giang and Hall buffer + 1 ml. of macerated and strained lemon fruit pulp.

workers have claimed that the departure from linearity is not sufficient to introduce significant errors into the subsequent calculations, and this is confirmed by curve *A* of Figure 3.

There appears to be no published discussion on the extent to which substances naturally present in plant extracts can interfere in this method by virtue of their buffering action. The rate of pH change with time is influenced by the effect of pH on buffer strength, and this can be isolated from the effect of enzyme activity by carrying out a potentiometric titration of the buffer. Figure 1 shows the titration curves of the original Michel buffer, of the Giang and Hall modification, and of the latter in the presence of macerated and strained lemon fruit pulp, and Figure 2 shows the pH against time graph obtained when the same three buffers are incubated with cholinesterase and acetylcholine. With Giang and Hall buffer there is a slight increase in sensitivity as the pH falls, due to a decreasing buffering action, but in the corresponding pH-time curve this is offset by a decrease in enzyme activity which progressively develops as the pH falls below 7, resulting in a graph which is a tolerably good straight line. It is evident from these figures that the sensitivity attainable with the Giang and Hall method, because of the higher buffer strength used, is considerably less than is obtained with the Michel method, yet this loss of activity is an advantage in that it overcomes the effect of small

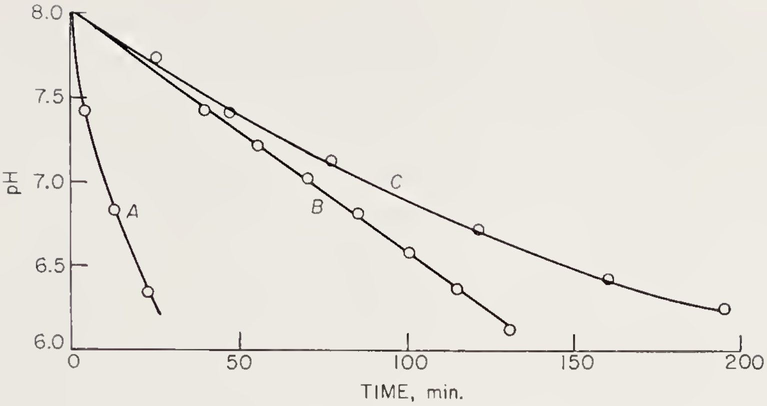


Fig. 2. Rate of pH change due to enzymic hydrolysis of acetylcholine. Curves *A*, *B*, and *C* correspond to buffers in Figure 1.

amounts of buffer in the plant extract. Nevertheless, when much buffer is present, as in neutralized lemon pulp juice, a loss of sensitivity and linearity occurs, as shown in curves *C* of Figures 1 and 2. In Figure 3 are shown the ΔpH values for a range of concentration of horse serum using the Giang and Hall buffer, and these points fall

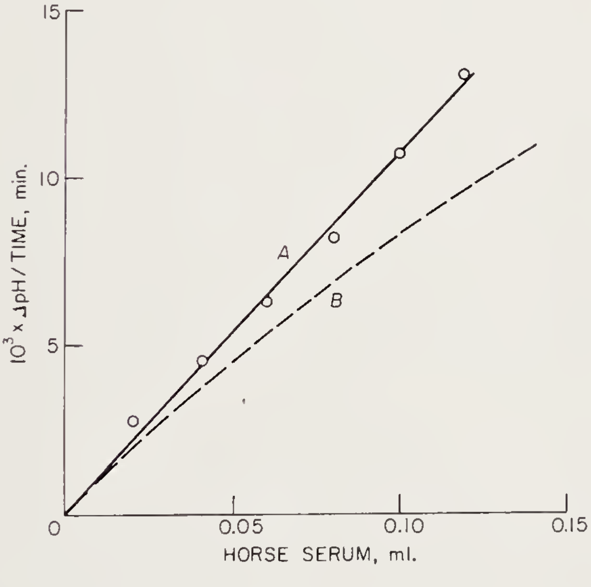


Fig. 3. Relation between pH change per unit time and volume of horse serum used. Curve *A*, experimental conditions as defined by Giang and Hall; curve *B*, calculated from curve *A*, assuming the presence of 1 ml. of lemon juice with a buffer strength as shown in Figure 1, curve *C*.

tolerably well on a straight line. The broken line in Figure 3 is the calculated ΔpH-activity curve derived from the potentiometric titration curve of the buffered lemon extract shown in Figure 1; although departure from linearity in this graph is not marked, the slope indicates the considerable loss of sensitivity resulting from the increased buffering action of the solution.

In the calculation of the percentage inhibition or residual activity of a cholinesterase preparation after treatment with an inhibitor contained in a plant extract, the measured activity of the inhibited enzyme must be referred to the initial or control activity of the enzyme before inhibition. On account of the interference which may arise from plant extractives, it may not be sufficient to use for this control enzyme activity a mixture of enzyme and buffer alone, as recommended by Giang and Hall. Hensel and his colleagues (15), in their modification of the Giang and Hall procedure, used for the control activity measurement, an extract from a crop untreated with pesticide but otherwise the same as that undergoing residue analysis. Such a procedure is to be preferred, but it may not be practicable if no identical but untreated plant material is available, as may be the case with the analysis of imported crops for the purpose of public health control. An alternative procedure is based on the principle, discussed more fully in Section III, that the effect of an inhibitor on an enzyme is largely neutralized if the substrate is added before the enzyme. By this means a control activity measurement may be made on a solution containing the plant extract, and the effect of extractive will be the same in this measurement as in the subsequent determination of activity after interaction of the enzyme with the plant extract.

It is customary to adjust the buffered plant extract so that the initial pH reading is in the region of 8, as the activity of the enzyme remains constant over the range pH 8–7 and only begins to fall off markedly below pH 6. For this reason a range pH 8–6 is taken as the maximum for measurement. It is evident from what has already been discussed that the timing of the initial and final pH measurements must be carefully controlled with a stopwatch. It is not necessary that the zero time reading should correspond with the mixing of the enzyme and substrate, but the reading should not be long delayed as this would limit the range of pH available for measurement.

It is desirable that the initial pH values of all solutions being investigated should be measured independently and not be assumed from a measurement on a single solution, as recommended by Giang and Hall (11).

In order to obtain reproducible results the cholinesterase activity measurement should be made at constant temperature. It is customary to maintain the solutions in the region of 37°C., although a lower temperature, which will involve a somewhat lower cholinesterase activity, may also be used. Even at the higher temperature the non-enzymic hydrolysis of acetylcholine will not significantly interfere unless very low enzyme activities, which require lower than usual buffer concentrations, are being measured. It has been recommended that the solutions be measured into 5 ml. beakers which are placed on a shelf in a constant temperature bath; a magnetic "flea" may be placed in each beaker to promote mixing, although this is not essential. Alternatively the beakers may be closed with aluminium foil and placed in a constant temperature oven. At accurately timed intervals the beakers are removed from the water bath, and a small electrode and a calomel reference electrode inserted; the publicity literature of various manufacturers gives several designs of microelectrodes suitable for this purpose. In the author's laboratory the solutions for measurement are placed in small test tubes in a water bath, and a mobile electrode system protected by a glass sheath, as described in Section II.C.2, is inserted at timed intervals into the tubes without removing them from the water bath.

It is also possible to follow the change in pH of the solutions by instrumental measurement of the optical density at selected wavelengths after the addition of a suitable indicator. Although such an operation dispenses with the need for a sensitive pH meter, it has no advantage and may be subject to interference from the presence of natural coloring matter.

2. Titrimetric Method

One of the earliest methods of measuring cholinesterase activity was the titration of the acetic acid liberated by hydrolysis of acetylcholine. This procedure has been elaborated by various authors (3); ideally, the alkali should be continuously added to the enzyme-substrate mixture to maintain a constant pH, for which purpose an apparatus of the type known as a "pH-stat" is used (17). Such a procedure would not be suitable for the multiple determinations of cholinesterase activity which are required in residue analysis; it is more practical to allow the pH to fall from a fixed initial value and, after a measured time interval, to return the pH to the initial value by means of the measured addition of alkali; this gives a direct measure of the rate of hydrolysis of acetylcholine if it is known that under these conditions the rate is constant.

The titrimetric method has been routinely used in the author's laboratory for a number of years to determine blood cholinesterase in a variety of animal species, and it has been adapted to residue analysis. It has an advantage over the pH change method in that the measured activity is not directly influenced by the buffer strength of the solution. The buffer can influence the precision of the initial and final pH adjustments by its effect on the rate of change of pH with addition of alkali, and to attain the maximal sensitivity of the method it is desirable that the buffer strength of the solution should be weak in the region of pH 8, which is taken as the initial pH of the solution. On the other hand, the solution should not be so weakly buffered that the pH can fall much below 7.5 during the period of acetylcholine hydrolysis. Ideally, the buffer should be weak at pH 8 and become stronger toward pH 7, and for cholinesterase assays on blood a $0.01M$ maleate buffer has been found satisfactory. In residue analysis there is usually sufficient buffer present in the plant extract to prevent an excessive pH fall; if not, then it is desirable to include at least $0.005M$ phosphate in the solution.

It is not necessary or desirable in residue analysis to make full use of the high sensitivity of the titrimetric method, for when low enzyme activities are being measured interference may arise from two factors which can otherwise be disregarded. Acetylcholine undergoes a slow hydrolysis in the absence of enzyme at a rate which depends on the pH and temperature of the solution, and in weakly buffered solutions the titrimetric method is sufficiently sensitive to measure this nonenzymic hydrolysis. The other factor is the influence of atmospheric carbon dioxide. When a solution at pH 8 in equilibrium with atmospheric carbon dioxide is allowed to become more acid, carbon dioxide will be liberated, and the amount of alkali required to return the solution to its initial pH will depend upon the extent to which the solution reaches equilibrium with the atmosphere at the lower value. At the buffer concentrations normally encountered in plant extracts, and with a de-

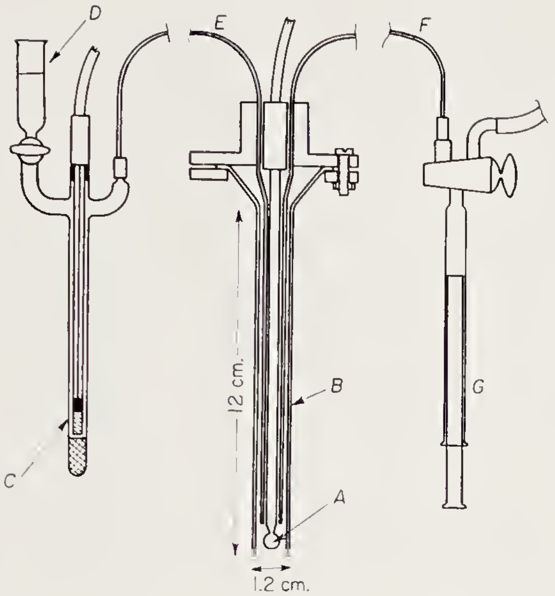


Fig. 4. Micro titration assembly. *A*, glass electrode bulb; *B*, glass sheath; *C*, calomel electrode; *D*, potassium chloride reservoir; *E* and *F*, polyethylene cannulae; *G*, titration syringe (micrometer head not shown).

fined method of carrying out the procedure, this effect of carbon dioxide can be neglected.

A convenient apparatus for micro titration is shown in Figure 4. The glass electrode has a bulb 0.5 cm. in diameter and a glass stem 14 cm. long. It is held in a polymethyl methacrylate head and is protected by a glass sheath, (1.2 cm. o.d.) pierced near the open end with holes to permit the solution to circulate round the electrode bulb. A saturated calomel reference electrode is connected to the glass electrode system by means of a long flexible polyethylene cannula (1 mm. o.d.), containing saturated potassium chloride solution, which can leak from the tip only when the tap of the reservoir on the calomel electrode is opened. A titration syringe filled with standardized sodium hydroxide solution and operated by means of a micrometer head is also connected by cannula to the glass electrode assembly. After each titration the glass electrode is placed in a tube and flushed with distilled water, and the tap on the potassium chloride reservoir is momentarily opened to form a fresh junction at the tip of the cannula.

The solutions to be investigated are introduced into tubes, sufficiently large to permit entry of the glass sheath, which are maintained in a water bath at 25°C. For measuring low inhibitor concentrations 4 ml. of plant extract is incubated with 1 ml. of 2% horse serum or an equivalent enzyme solution for 30 minutes; then 0.2 ml. of $0.1M$ acetylcholine is added and the pH is adjusted to 8.0. At a timed interval after this pH adjustment, usually in the region of 60 minutes, the pH is again brought to 8.0 and the amount of sodium hydroxide solution used is recorded. With horse serum about 0.04 ml. of $0.025N$ sodium hydroxide, or 1 μ mole, is required for a hydrolysis period of 1 hour. Under the same conditions the nonenzymic hydrolysis of acetylcholine amounts to about 0.05 μmole, sufficiently large to justify a correction being made during activity measurements to ensure that the measured liberation of acetic acid is proportional to the enzyme activity. The application of this correction may be avoided by using a stronger enzyme solution with a correspondingly stronger sodium hydroxide solution and a higher buffer concentration.

3. Measurement of Unchanged Acetylcholine

The measurement of cholinesterase activity by following the disappearance of the substrate acetylcholine permits the use of a colorimetric procedure, which, though somewhat more complicated than the potentiometric or titrimetric determination of acetic acid, can be used when the accurate pH meter required for these methods is not available. The instrument used for the colorimetric determination of acetylcholine need not be complicated, and under primitive conditions when high accuracy is not required a visual matching with color standards may be adequate.

This type of method has been applied to residue analysis by Cook (5), although his theoretical treatment is not to be recommended. He determined the unchanged acetylcholine by the method of Hestrin (16), in which it is reacted with alkaline hydroxylamine to produce acetohydroxamic acid, which yields a purple color with excess ferric chloride after acidification. The alkaline hydroxylamine is added to the enzyme-inhibitor mixture after the appropriate incubation period; this halts the enzyme action and precipitates protein together with other constituents of the plant extract, including most coloring matter. After filtration excess ferric chloride and hydrochloric acid are added, and the color which develops is measured at a wavelength in the region of 540 mμ. In the presence of the excess yellow ferric ion the purple color of the ferric acetohydroxamate appears brown. As the activity determination involves a measurement of the decrease in the acetylcholine concentration over a measured hydrolysis period, the initial acetylcholine concentration should be as high as possible in order to gain greatest sensitivity and range, but this is limited by the highest optical density reading which can be achieved with the instrument used. It is not usually possible to exceed a concentration of $0.005M$. The optical density of the color produced is proportional to the acetylcholine concentration, and if the instrument used does not introduce any departure from this linear response, an increased range may be obtained by comparing the colors produced from the solutions before and after hydrolysis, zeroing the instrument with the latter and then reading the former.

Esters of carboxylic acids, and certain other substances which may be present in plant extracts taken for residue analysis, can yield a purple color under the conditions of this method. By the inclusion of a suitable blank determination on the extract an interference from this source can be avoided. However, if the effect is large, it should be eliminated by means of a cleanup procedure, or the optical densities encountered will be too high for precise measurement.

It has been shown in equation 3 that under the conditions of this method enzyme activities cannot be expressed as a simple function of the rate of change of substrate concentration. Enzyme activity measurements are most simply achieved by first preparing a standard curve relating known amounts of enzyme to the measured change in substrate concentration, keeping the initial concentration and the hydrolysis period constant. Provided that the substrate concentration is not allowed to fall much below one-half its original value, the departure of this curve from linearity will not be large. The series of enzyme solutions to be used for preparing this standard curve should be adjusted to a uniform pH, preferably 7.5–8.0, and 2 ml. volumes are pipetted into tubes held at 37°C. in a water bath. At a measured time 2 ml. of $0.01M$ acetylcholine is added to each, and exactly 15 minutes later 2.5 ml. of alkaline hydroxylamine, prepared by freshly mixing equal volumes of 20% (w/v) hydroxylamine hydrochloride and 20% (w/v) sodium hydroxide solutions is added. After at least 1 minute 3 ml. of a 10% (w/v) solution of hydrated ferric chloride ($FeCl_3 \cdot 6H_2O$) in a diluted hydrochloric acid, prepared by mixing equal volumes of concentrated acid (sp. gr. 1.18) and water, is added. The solutions are filtered if not clear, and about 30 minutes after the addition of the ferric chloride reagent each solution is transferred to a 1 cm. cuvette for optical density measurement at 540 mμ. At the same time, to 2 ml. of the enzyme solution is added 2.5 ml. of alkaline hydroxylamine simultaneous with, or a little prior to, the addition of acetylcholine, and the procedure completed as above; this treatment gives the color due to the unchanged acetylcholine concentration, S_0. In the preparation of the standard curve the optical density of this solution is read with the instrument zeroed against each solution prepared from known enzyme concentrations, the readings giving a direct measure of the change in substrate concentration, ΔS, if a suitable instrument is used. For residue analysis a standardized enzyme preparation is required, and from the information derived from the standard curve the enzyme is diluted so that the optical density (equivalent to ΔS) for 1 ml. is 0.5 ± 0.03. This standardization should be checked each day.

The standard curve for residue analysis may relate the measured optical density difference (= ΔS) to the inhibitor concentration. Into a series of tubes are measured 1 ml. of standardized cholinesterase preparation and, at timed 1 minute intervals, 1 ml. of a series of inhibitor concentrations. After exactly 30 minutes, the substrate is added and the procedure completed as above. In the assay of a plant extract two 1 ml. portions are incubated for 30 minutes with 1 ml. of enzyme each; the substrate is then added to one, followed by a 15 minute incubation before the reaction is halted; to the second the alkaline hydroxylamine is added a little before the acetylcholine. The measured difference between the optical densities of the two resultant solutions after color development, which is a measure of the unchanged acetylcholine not subject to error from small amounts of natural color-producing substances in the plant extract, is used to calculate the inhibitor concentration from the standard curve. It is desirable that all determinations should be made in duplicate and the averages used.

This method has been used for residues of amiton in orange and lemon peel, in the range 0.05–0.3 p.p.m., and the results obtained were in excellent agreement with those obtained by radioactivity assay (20).

4. Other Methods

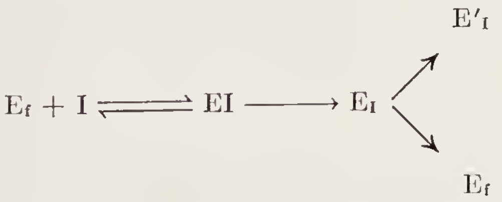
The manometric determination of cholinesterase activity, in which the carbon dioxide liberated from a bicarbonate buffer by the hydrolysis of acetylcholine is measured in a Warburg or similar apparatus, has been extensively used to study the location and the properties of the enzyme. As the techniques and apparatus are more complex than those required for other methods, the manometric method does not appear to have been widely used for residue determination by cholinesterase inhibition, and it is not, in general, to be recommended for this purpose. Details of the variations in this type of method are discussed by Augustinsson (3); the method involves essentially the maintenance of a constant pH, and hence, like the titrimetric method, is not greatly influenced by buffers present in a plant extract.

A number of colorimetric methods for measuring cholinesterase activity have been described which require a special substrate, itself colorless but capable of being broken down by enzymic hydrolysis to yield a colored product, or a substance from which a colored derivative may easily be prepared. Examples of such substrates are indophenyl acetate, β-naphthyl acetate, indoxyl acetate, and acetylthiocholine; the use of these has been reviewed by Augustinsson (3). Methods of this type do not appear to have been used for residue analysis, but they are likely to be suitable, provided that substances present in the plant extract do not interfere with the colorimetric determination.

III. Inhibition of Cholinesterase by Organic Phosphate Compounds

A. KINETICS OF INHIBITION

A scheme for the reaction between cholinesterase and an organic phosphate ester, analogous to that shown above (eq. 1) for the enzyme-substrate reaction, can be written:



$$\begin{tikzcd} & & & E'_I \\ E_f + I \arrow[r, shift left] & EI \arrow[l, shift left] \arrow[r] & E_I \arrow[ur] \arrow[dr] & \\ & & & E_f \end{tikzcd}$$

(4)

Here the free enzyme, E_f, and the inhibitor, I, form a reversible enzyme–inhibitor complex, EI. However, according to the current hypothesis (1), the substituted phosphoric acid produced from the

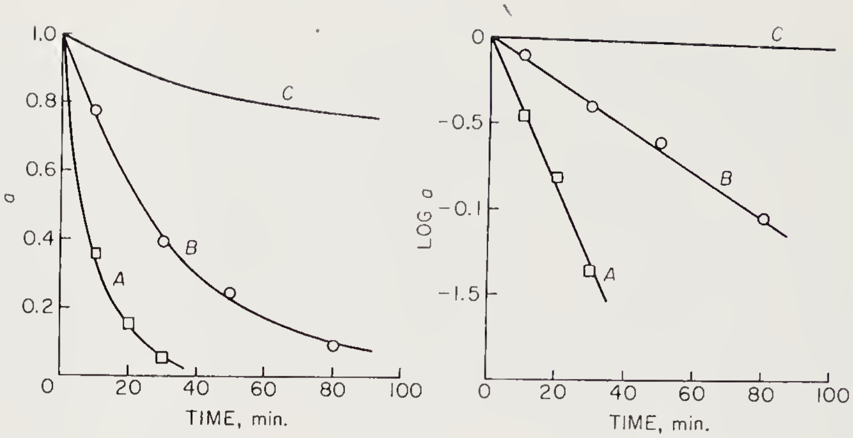


Fig. 5. Rate of change of fractional residual activity, a, of horse red cell cholinesterase in presence of inhibitors at 25°C. Curves A, $10^{-7}M$ paraoxon curves B, $10^{-7}M$ amiton curves C, $10^{-7}M$ amiton with $5 \times 10^{-3}M$ acetylcholine (continuous curve obtained from "pH-stat" apparatus).

complex as one of the hydrolytic fission products is not released but reacts with an active center of the enzyme. The inactive phosphorylated enzyme, E_I, so produced can either slowly hydrolyze to revert to the active enzyme or be slowly converted by a transphosphorylation process to an irreversibly inhibited enzyme, E'_I. It is believed that the rate constants of these reactions are such that at no time is there any significant amount of the complex, EI, present, and it can be shown experimentally that the rate of regeneration of the free enzyme is very much slower than the rate of inhibition. For the purpose of residue analysis the reaction may be simplified to the irreversible form with the rate constant K_3:

$$E_f + I \xrightarrow{K_3} E_I \tag{5}$$

From the reaction of cholinesterase with physostigmine Goldstein (12) has shown that, at measurable rates of inhibition, the concentration of inhibitor is very much greater than that of the active centers of the enzyme and is not significantly altered in the course of the reaction. This has been confirmed in the author's laboratory for paraoxon (diethyl p-nitrophenyl phosphate) and amiton (diethyl S-β-diethylaminoethyl phosphorothioate), and it may be assumed to hold for all organic phosphate and carbamate inhibitors. Curves A and B of Figure 5 show that the inhibition follows the expected course given by equation 6.

$$E_f/E_0 = a = \exp(-K_3 I t) \tag{6}$$

or

$$\log_e a = -K_3 I t$$

where a is the fractional residual activity or the ratio of the concentration of free enzyme after time t to the concentration of the initial enzyme, E_0. If substrate is present during the inhibition period, it competes with the inhibitor for the active centers of the enzyme, and it can be shown that the course of the reaction is then given by equation (Fig. 5, curve C):

$$a = \exp(-K_2 I t K_S/S) \tag{7}$$

or

$$\log_e a = -K_3 I t K_S/S$$

At the concentrations of organic phosphate inhibitor and substrate which are practicable in residue analysis no equilibrium occurs, and the inhibition proceeds to completion if left long enough. The rate of the inhibition is defined by the values of the constant K_3, which for paraoxon and amiton with horse red cell cholinesterase are 9.75 and 2.9×10^5 liters/mole/minute, respectively, at 25°C. The more usual figure for defining inhibitory activity is the I_{50}, or the concentration of inhibitor which will produce a 50% inhibition, but this is a function not only of temperature and enzyme source but also of the duration of the inhibition; from equation 6 it can be shown that the I_{50} is inversely proportional to t. The 60 minute I_{50} values for the K_3 values quoted above are 3.12×10^{-8} and $1.05 \times 10^{-7} M$, respectively, and it is probable that all direct *in vitro* inhibitors of cholinesterase will have values of this order. Organophosphorus compounds, which are only weak *in vitro* inhibitors, as exemplified by the commercially important range of phosphorothionates, must be suspected as being substances with little or no direct activity and possibly contaminated with more active substances (8). Methods of dealing with these indirect cholinesterase inhibitors are described in Section V.

B. PRACTICAL APPLICATION

From equation 6, it may be seen that the inhibitor concentration, I, can be obtained from a plot of $\log_e a$ against t, if the rate constant, K_3, is known with adequate accuracy. Such a procedure would be too cumbersome for the many determinations usually required in residue analysis; moreover, the magnitude of the rate constants is rarely available, and such an absolute method might involve a systematic error if substances present in the plant extract influenced the measured enzyme activity. A more usual and convenient procedure is to measure the activity of an enzyme preparation before and after incubation with the inhibitor solution for a fixed time and temperature. From equation 6 it is seen that if t is constant, log a is proportional to the inhibitor concentration, and that a series of experiments under standard conditions, in which the enzyme is incubated with known inhibitor concentrations, will enable a linear standard curve to be constructed by means of which the measured residual activity may be converted to the concentration of inhibitor present, provided that the conditions of the experiment are identical with those operative for the standard curve. In the preparation of the standard curve it is desirable that the solutions should contain an extract from a control crop similar to that to be used for residue analysis, but not subjected to pesticide treatment.

In view of the theoretical relation between log a and I, which is also supported by practical experience, it is surprising that Giang and Hall (11), who were largely responsible for the introduction of residue determination by cholinesterase inhibition assay, elected to plot the logarithm of the inhibitor concentration against percentage enzyme inhibition in the preparation of a standard curve, and this practice has been followed in other modifications of the method. On the basis of equation 6 such a graph might be expected not to be linear, and this is found in practice, although the experimental errors do not permit the deviations from linearity to be observed over the central portion of the curve. Figure 6 shows a Giang and Hall plot of experimental results obtained with amiton and its transformation to log a against I. The latter graph has the great advantage that theoretically it should be a straight line passing through the origin; if the experimental graph is otherwise, either the measurements are not proportional to enzyme activities or there is some interference from a component of the plant extract. It is, therefore, capable of yielding more information on the nature of the inhibition, and is preferable to the Giang and Hall procedure.

A standard curve according to Giang and Hall has recently been published by Boyd (4) for *O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothionate. This curve and its transformation, as discussed above, are shown in Figure 7. It will be seen that the transformed

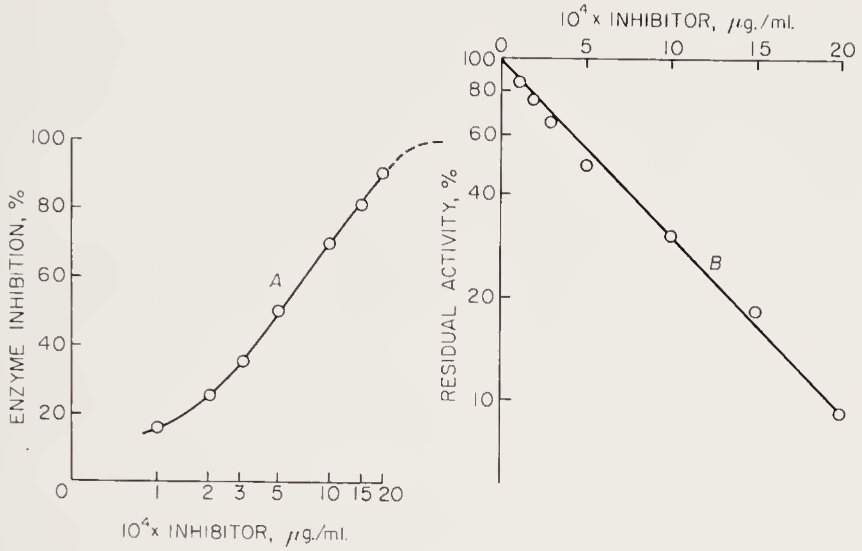


Fig. 6. Experimental relation between enzyme activity and inhibitor concentration. Results obtained using micro titration method with horse red cell cholinesterase and amiton oxalate, incubated for 60 minutes at 37°C. *A*, Results plotted according to the method of Giang and Hall; *B*, same results presented according to equation 6 given in text.

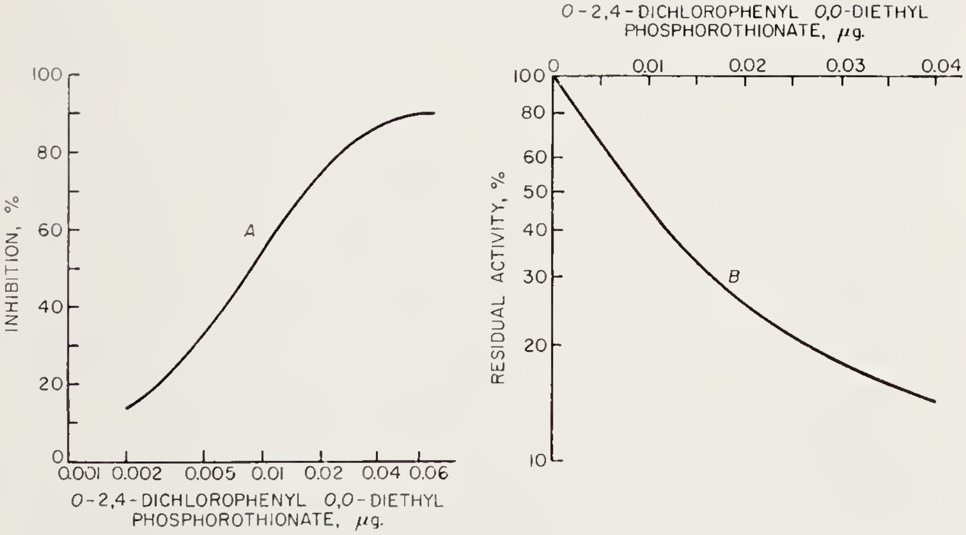


Fig. 7. Experimental relation between enzyme activity and inhibitor concentration. *A*, derived from curve published by Boyd (4); *B*, the same curve transformed as described in the text.

curve when extrapolated passes through the origin and is linear to a residual activity of about 40%. At lower activities the curve progressively departs from linearity, suggesting that the pH change method of activity measurement which was used does not give a response proportional to enzyme activity at these lower values.

It has already been stated that at measurable inhibition rates the concentration of inhibitor is much larger than that of the active enzyme centers and that if the reaction is allowed to proceed long enough, inhibition will become complete. For the most accurate results the percentage inhibition should not be less than 20 or more than 80, and the period of inhibition selected should be such that the material to be analyzed gives a figure within this range. With low enzyme activities and long inhibition periods any traces of inhibitory substances naturally present in the plant extract will exert a greater influence, and it may be necessary to concentrate and purify extracts with low inhibitor concentrations in order to remove interference. The termination of the inhibition period is taken to be the addition of the substrate, and this addition should be accurately timed. Although it is frequently stated that the inhibition ceases after the addition of the substrate, equation 7 shows that this is not strictly true, but that the rate of inhibition is greatly diminished. With horse serum cholinesterase with a Michaelis constant in the region of 1.2×10^{-3} mole/liter the addition of acetylcholine to bring the final concentration to $0.06M$ reduces the rate of inhibition to one-fifth. This residual inhibition need not be taken into consideration if identical methods of cholinesterase activity measurement are used throughout.

The determination of residues by cholinesterase inhibition analysis involves the serial addition of enzyme to the extracts at timed intervals, followed by the timed addition of substrate. The operation to measure the residual activity is also made at timed intervals; the intention being that each solution should have the same period of contact between enzyme and inhibitor, and the same period of substrate hydrolysis. The interval between these timed additions will be determined by the most complex operation involved; this is usually the final measurement of residual activity. It is rarely necessary for this interval to be greater than 2 minutes, and it can often be reduced to 1 minute; thus, with incubation and hydrolysis periods of 1 hour a considerable number of solutions can be dealt with concurrently.

As the inhibitor concentration is not significantly changed during the course of an inhibition, the initial enzyme concentration should not influence the magnitude of the percentage residual activity produced by a given inhibitor concentration, or the slope of the standard curve, although it will, of course, affect the precision of the activity measurement. The source of the enzyme may also have some effect on precision if there is a measure of specificity in the enzyme-inhibitor reaction; in order to obtain maximal sensitivity it may be necessary to select the type of cholinesterase with which the inhibitor has the greatest reactivity.

Other factors than time will influence the progress of the inhibition. The temperature coefficients of the reaction of a few organic phosphate insecticides with cholinesterase have been measured and have been shown to be of a magnitude consonant with a chemical reaction between enzyme and inhibitor (1), and it is probable that this applies to all members of this group (2). The temperature of the solution during inhibition should, therefore, be accurately controlled, preferably to within 1°C. The temperature selected for the inhibition is usually in the region of 37°C., although a lower temperature may be used if desired. The effect of pH is less certain. There are a few cases of organic phosphates whose activity varies according to the state of ionization; thus amiton contains a tertiary amino group which confers activity to the molecule only when it is positively charged, and above pH 8.5 the increase in the amount of nonionized form present brings about a decrease in the inhibitory activity (23). The effect of pH of the physical state of the enzyme may also influence the rate of inhibition. It is customary to adjust plant extracts to a fixed pH, usually in the region of 7.5, before adding the enzyme and inhibitor. However, when the pH change method of activity measurement is used, a strongly buffered extract at pH 7.5 may lower the initial pH below 8, and it may be desirable to carry out the inhibition incubation at a pH nearer this latter figure.

IV. Preparation of Sample for Analysis

The procedures necessary to ensure that the sample taken for analysis is truly representative of the material as a whole have been adequately reviewed elsewhere (13) and will not be discussed here. With few exceptions the sample must be processed before it is in a suitable form for inhibition analysis, and the extract thereby produced must contain all the inhibitor in the sample, without including other substances which might significantly interfere at some stage of the analysis.

It is not certain whether the rate of inhibition of cholinesterase by organic phosphates can be increased or decreased by substances naturally occurring in plant extracts, although anomalous results obtained with some materials such as alfalfa have suggested that this is not impossible. Such an interference would cause a change in the slope of the graph relating the logarithm of the residual activity to the inhibitor concentration, although the line would still pass through the origin. Traces of substances with an inhibitory action on cholinesterase are not uncommon in plant extracts; if present in significant amounts these would cause the graph to form an intercept on the concentration axis without altering its slope. To avoid such interference it is desirable, whenever possible, to carry out the tests on which the standard curve is based in a manner similar to that used for the treated sample, using an extract from untreated control plant material of similar origin to which have been added known amounts of the insecticide. This procedure will overcome the interference from substances which cause a change in the slope or position of the standard curve, provided that this interference is not too large; the standard curve should be prepared afresh if new material is being examined or a new source of enzyme used. If, as in the public health control of treated crops, a sample of untreated control material is not available, a cleanup procedure of proven efficiency must be used to separate the insecticide from natural inhibitors. An interference causing a change in the slope of the graph can be overcome by fortifying the extract, that is to say, by adding a series of known amounts of insecticide to it in the manner used to prepare the standard curve; when the graph relating logarithm of residual activity to inhibitor concentration is extrapolated to cut the concentration axis, the intercept gives the total inhibitor content of the solution, together with any natural inhibitors present. When natural inhibitors or other substances are present in the plant extract to such an extent that they no longer permit an analysis of adequate accuracy and precision, the extract must be submitted to some purification process. Any of the methods used for cleanup prior to chemical analysis may be applied and as these have been the subject of an excellent review (13), they will not be considered in detail here. The selection of the method will depend upon the chemical natures of the pesticide and of any natural interfering substances and upon the method used to measure cholinesterase activity. With methods which involve the measurement of a pH change the elimination of organic acids which may buffer the solution over the operative range may be necessary, while colorimetric methods will be affected by colored substances in the extract or by substances which develop a color under the conditions of the test.

The inclusion of a purification procedure such as solvent extraction raises a problem which is common to all forms of residue analysis and will only be touched upon here. To what extent is it possible to be sure that all the residue in the sample is being extracted by the procedure used? Large organizations may be able to justify their analytical procedure by treating a crop with the pesticide labeled with a radioactive isotope, but this is not always possible, particularly in the public health control of treated crops. Another recommended procedure is the isotope dilution technique (24). Repeated extraction of the sample may yield information on efficiency, but this will not help if a portion of the residue is firmly bound to some plant constituent. Probably the most generally applicable procedure, if the intention is to determine whether the pesticide in a crop is within an established tolerance, is the fortification procedure mentioned above. The sample is homogenized in water, and to portions are added increasing amounts of the pesticide. Each portion is subjected to purification and subsequent analysis, and the logarithms of the residual activities obtained are plotted against the concentration of added inhibitor; extrapolation of the graph to the inhibitor axis will yield the initial inhibitor concentration. If this value does not exceed the prescribed tolerance, the crop is satisfactory; if it does, then an attempt should be made to obtain a sample of the untreated crop if there is any suspicion that natural inhibitors may be present.

The procedure which has been most widely used for purifying plant extracts prior to cholinesterase inhibition analysis is extraction with chloroform, which will efficiently remove the great majority of commercially important insecticides. The chloroform is cautiously evaporated off and the residue dissolved in water. An extension of this procedure has been used for amiton; on account of the cationic group present in this molecule the residue can be extracted from an organic solvent by shaking with dilute acid, thereby avoiding the removal of the solvent.

Although purification is not generally so important for cholinesterase inhibition analysis as for chemical analysis, it occasionally makes possible the separation of different organic phosphates. There is another factor which must be considered when cleanup methods are being developed for new pesticides. Organic phosphates are known which undergo metabolism in plant tissue to substances with different chemical properties but with a cholinesterase inhibitory activity and a mammalian toxicity at least as high as those of the parent substance. An example of this is the oxidation of systemically absorbed demeton to sulfoxides and sulfone derivatives (19). In such cases an increased efficiency of purification of the parent substance may result in an increased loss of a component which contributes largely to the toxic residue on the crop. This may seriously interfere in chemical methods of analysis. By inhibition analysis on the other hand, potentially toxic derivatives can be detected even when they have undergone a considerable chemical change. The establishment of an analytical method for residues of a new organic phosphorus compound must be preceded by metabolism studies in which inhibition analysis will play an important part.

V. Activation of Cholinesterase Inhibitors by Oxidation

The application of cholinesterase inhibition methods to residue determination is limited to those organic phosphates which have a direct *in vitro* action on the enzyme. There are, however, many members of this group, and among them the majority of the commercially important insecticides, which produce an *in vivo* inhibition of cholinesterase in insects and in mammals, yet which have little or no effect on the enzyme *in vitro*. Giang and Hall (11) were the first to show that the weakly inhibitory parathion could readily be converted in good yield, by means of fuming nitric acid, to a powerful cholinesterase inhibitor. They assumed that the parathion was converted to its oxygen analogue, paraoxon, and they suggested that this procedure could be used for the determination of parathion residues in crops. Fallscheer and Cook (9) showed that the activation of parathion could more easily be achieved by means of bromine water, and they produced evidence to show that the parathion was being converted to paraoxon. They also showed that a number of phosphorothionates could be converted to potent cholinesterase inhibitors by this method, presumably by a similar oxidation of a P=S to a P=O group. It is interesting that the weakly inhibitory phosphorothionates undergo a similar oxidation by enzymes located in various tissues (10), and without doubt their powerful *in vivo* inhibition of cholinesterase is due to this biological metabolism. The reagent used by Fallscheer and Cook was saturated bromine water diluted 0.4 to 100 with water; 0.1 ml. of this added to 0.9 ml. aqueous insecticide solution produced an instantaneous conversion in an almost quantitative yield. These authors claimed satisfactory results with parathion, methyl parathion, Chlorthion,* malathion, Diazinon, and EPN, and in preliminary experiments encouraging results had been obtained in the recovery of these substances added to plant extracts, though in some cases it was found necessary to increase the amount of bromine to effect the oxidation. Cook (7) has stated that further experience has shown that the method is not suitable for sulfatepp and the phosphorothionate isomer of Systox* and that difficulties are occasionally encountered with Diazinon and EPN.

Certain phosphorothionates which cannot be activated by bromine in the cold can be oxidized by peracetic acid under more severe conditions. This method was introduced for Trithion** (18) and has been successfully used for Thimet*** and Di-Syston* (22). The reagent is a freshly prepared mixture of 30% hydrogen peroxide and glacial acetic acid, 5 ml. of a benzene extract of plant tissue is heated with 3 ml. of this reagent for 20 minutes at 75°C. The benzene is then washed with water, measured portions are evaporated to dryness, and the residue dissolved in water.

Nitric acid has been used by Boyd (4) to oxidize *O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothionate. His oxidizing acid is a mixture of equal volumes of fuming nitric acid (sp. gr. 1.50) and concentrated nitric acid (sp. gr. 1.42). A purified chloroform extract of the sample is prepared and 5 ml. of this is evaporated in a 250 ml. flask; 5 ml. of oxidizing acid is added and left for 10 minutes. The solution is diluted and the excess acid neutralized with sodium bicarbonate, and an ether extract of the oxidized product is prepared for cholinesterase inhibition assay.

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* Registered trademark Chemagro Corporation, Kansas City, Mo.

** Registered trademark Stauffer Chemical Co., New York, N. Y.

*** Registered trademark American Cyanamid Co., New York, N. Y.

The application of cholinesterase inhibition methods for the determination of indirect inhibitors is of little value. Not only does the low activity require a large sample to be used, but it is very difficult to be certain that the activity is in fact a true property of the material and not due to traces of an active inhibitor present as impurity. In the latter case the activity of the insecticide might vary from batch to batch, or the residue in the crop might undergo an apparent change in inhibitory activity if the metabolic fate of the impurity differed from that of the main constituent.

VI. Applications of Cholinesterase Inhibition Analysis

The great advantage of cholinesterase inhibition methods for the determination of organophosphorus residues is that they measure a specific configuration of the molecule which, with a high degree of probability, is responsible for the toxic action toward insects and mammals, and with a sensitivity which at its best is far greater than that obtainable by chemical analysis. This form of specificity renders the methods particularly suitable when the insecticide undergoes changes in the plant to produce metabolites with a high inhibitory activity, as demonstrated by the complex fate of demeton and its analogues, which give rise to sulfoxides, sulfones, (19) and possibly also to sulfonium compounds (14). In such cases residue analysis by chemical methods would be a formidable task and an important metabolite might well be missed unless inhibition analysis were used as a check.

In spite of this configuration specificity, which enables an active inhibitor to be distinguished from an inactive metabolite, inhibition methods are frequently criticized for lacking the specificity to distinguish among different types of organophosphorus compounds. This criticism must be admitted, although for the extensive experiments undertaken by insecticide manufacturers to establish a tolerance figure for a new insecticide such a consideration is probably of little importance, as usually only one insecticide is involved. For the examination of crops which are known to have been treated with several insecticides, or for the investigation of a crop of unknown origin by a public health authority, this lack of specificity may be a disadvantage. In such cases it may be possible to classify the inhibitors present to some extent by physico-chemical separation or by oxidation to produce activation or to use a specific enzyme reaction. This criticism may also be countered by observing that few chemical methods for determining organophosphorus residues can themselves claim a high degree of specificity. Even the chemical determination of parathion based on the measurement of the aromatic amine produced by reduction of the *p*-nitrophenyl group is not free from possible error; after feeding to cows crops which had been treated with parathion, Cook (6) found the presence of parathion in the rumen contents by chemical analysis, but obtained a negative result by cholinesterase inhibition after bromine activation, this led to experiments which demonstrated the reduction of parathion in the rumen to the corresponding amino compound. Because many of the phosphorus insecticides at present being commercially exploited do not possess a characteristic group such as the nitrophenyl group of parathion, chemical analysis must have recourse to methods of low specificity such as the micro determination of phosphorus or sulfur.

It is evident from the previous sections that the methods used in cholinesterase inhibition residue analysis differ considerably from the more usual chemical procedures, and it is possible that some of the difficulties which have been encountered by analysts in applying these methods are due to their failure to acquaint themselves with the nature of the reactions which they are using and with the special precautions which are necessary to avoid error. There is now abundant evidence that, provided a method is selected which is appropriate to the insecticide and to the crop, reliable and reproducible results can be obtained by properly trained laboratory assistants.

A more serious criticism of these methods is that in their simplest form they are suitable only for those substances which have a direct inhibitory action on cholinesterase and are thus quite limited. Moreover, the use of such substances may well tend to decrease as commercial interest is centered more on insecticides with a low mammalian toxicity. Chemical oxidation, analogous to biochemical activation, of the large group of indirect inhibitors offers great promise of increased scope for these methods. Such oxidation procedures have been established as routine in some laboratories, but they do not seem to have yielded good results in the hands of all analysts. It may be that these methods have not been described in sufficient detail in the scientific literature or private communications to yield reproducible results in the hands of others or that they are strictly limited to the type of crop or to the particular insecticide for which they were designed. It seems likely that at the present time any attempt to apply these procedures to residue analysis must be regarded as a research project, and that the investigator must be prepared, if necessary, to study a variety of oxidizing agents under different conditions as well as to give careful attention to the fractionation of the crop sample.

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PRINCIPLES OF AQUATIC PLANT CONTROL

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I. Introduction

Plants play an important role in man's welfare in a number of ways. They provide raw materials for food, shelter, and clothing, either directly or indirectly through further synthesis in his ingenious industrial plants. Plants also exert an important influence in human affairs as part of our over-all environment which is simultaneously shared with other organisms. Over the years, the efforts of man have been directed toward the improvement of his environment through the development, growth, and promotion of desirable plants and associated organisms, at the expense of those inimical to his general welfare and well-being. In this respect, plants of the aquatic environment may be desirable contributors to man's welfare or may be undesirable by seriously interfering with his mode of life, or even with life itself. The planned management and control of aquatic plants, where feasible, may often contribute to his welfare. Ways in which such activities may be helpful include the diverse fields of public health, fisheries and wildlife management, navigation, irrigation, recreation, and others.

In the field of public health, aquatic plants may be responsible for the development of vectors of human disease and of insect pests which cause much discomfort and annoyance to man. In some cases, the interrelationships between aquatic plants and vectors of disease, and even insect pests, are well established. The close association between the aquatic stages of certain anopheline vectors of human malaria and aquatic and semiaquatic vegetation and flotage is an outstanding example of this interrelationship (39,42,69,80). Aquatic plants also are involved in schistosomiasis, an important disease of man in Africa, China, South America, and elsewhere. In Egypt certain aquatic snails, such as *Bulinus truncatus* and *Planorbis boissyi*, serve as the intermediate gastropod host of the causative trematode (6). The effective management and control of aquatic plants favorable to the intermediate host snail may contribute to the alleviation of human suffering. Aquatic plants, following strandage in windrows along shorelines, sometimes serve as breeding sites for pestiferous insects. For example, fermenting and decaying windrows of aquatic plants can produce large numbers of the stable fly (*Stomoxys calcitrans*), which viciously attacks man and his domestic animals (37). Aquatic plants also have other human health implications associated with water supplies, sewage disposal, and stream sanitation. Some aquatic plants, such as smartweed (*Polygonum hydropiperoides*), are causative agents of dermatitis. The wind-borne pollen of aquatic grasses may cause pollinosis or hay fever. It is interesting to note that the wind-borne pollen of cattail is notably inactive in this respect (81,103,104). In general, aquatic plants appear to be of only minor importance in relation to hay fever. In these and other ways, aquatic plants exert a real influence in the field of public health.

In the fields of fisheries and wildlife management of wetlands, the control of aquatic plants may often be an important item for increasing and maintaining these valuable resources (60,61). Higher aquatic plants, and sometimes excessive growths of algae, may be detrimental to fishes. These aquatic plants at certain periods may reduce the oxygen supply below the threshold level for fishes and invertebrate members of food chains. Excessive algal growths may also kill fish by clogging their gill chambers. Higher aquatic plants and mats of algae compete with desirable phytoplankters important for initiating desirable food chains for greater fish productivity. The water hyacinth (*Eichhornia crassipes*) has invaded extensive areas of open water in the southern part of the United States and causes much loss in our wildlife resources. Oxygen levels in water beneath mats of hyacinth were found to be below the threshold level for fishes (57). Heavy growths of algae have been known to maintain dissolved oxygen near the saturation value in the daytime even though, as a result of organic pollution, the same area might become almost completely devoid of oxygen at night (48). In such situations, the cause for the absence of fish populations would not be revealed by daytime sampling alone.

In wildlife management, wetlands contribute resources of much economic importance, and significant progress has been made in their management and in the control of undesirable aquatic plants. These areas are important in relation to our national waterfowl populations and to the production of valuable fur-bearing animals such as the muskrat. They also are of importance in supporting many other forms of wildlife. In relation to waterfowl, it was estimated that Americans spend over $100 million annually in harvesting the yield from this standing resource (60). The annual harvest of all pelts from the United States, including Alaska, was reported to exceed 8 million in number for the 1955–56 season (96). A number of aquatic weeds and a few species of water-tolerant woody plants are involved in the degrading of wildlife habitats. Examples from these two groups include such plants as water hyacinth (*E. crassipes*), alligator weed (*Alternanthera philoxerides*), water primrose (*Jussiaea diffusa*), black willow (*Salix nigra*), and buttonbush (*Cephalanthus occidentalis*).

Of all aquatic plants, it appears that water hyacinth (*E. crassipes*) is the most important in interfering with movement of boats through southern waterways. This floating-mat aquatic often chokes bayous, lakes, lagoons, canals, and other waterways, making boat passage impossible. As early as 1900, the U.S. Army Engineers initiated a control program against water hyacinth in order to keep the waterways open to boat traffic (91). Shifting rafts of this plant may often impede the return of sportsmen from a recreational area, causing considerable inconvenience and discomfort. In rare cases, movement may be prevented long enough to result in undue exposure, sickness, or death (78). Sometimes aviators have mistaken green areas of water hyacinth for landing strips, much to their own amazement, danger, and financial loss (45,78).

Aquatic plants impede the flow of water along natural drainage pathways, in irrigation canals, and in various types of ditches. Plants such as water hyacinth (*E. crassipes*), parrots-feather (*Myriophyllum brasiliense*), and others sometimes choke streams and impede runoff to the extent that the backwater buildup floods marginal lands. In extreme cases, aquatic weeds may reduce the flow as much as 97% (89). In one situation water hyacinth blocked a stream and sewage backed up into a populated area (78).

The control of aquatic and ditchbank plants associated with irrigation projects is also an important field of plant control (79). The objectives are to remove weeds from the ditch in order to maintain desired rates of flow, to prevent the introduction of weed seeds in crop lands, and to deliver to the field water which is not injurious to the crops. Plants of importance in irrigation ditches include tules (*Scirpus*), cattail (*Typha*), rushes (*Juncus*), and other emersed and submersed species, such as pondweeds (2,11,79). Aquatic plants are not a real problem in irrigation ditches lined with concrete (79); here only ditchbank species may need to be controlled in order to prevent infestation of crop lands with weed seed.

Some aquatic plants interfere with certain recreational activities of man. They oftentimes interfere with harvesting of fish and wildlife crops and may even ruin waters for fishes. Bathing sites may be made unsatisfactory by algal growths or unpleasant by the presence of the spiny caltrops of the water chestnut (*Trapa natans*) (59,66). Bathers may also be irritated by swimmers itch (10), which is due to the local and temporary irritation of the skin by cercariae or larval schistosomes which spend part of their life cycle in snails dependent upon aquatic vegetation (10). Aquatic plants may also be responsible for hordes of mosquitoes and other pestiferous insects which spend their aquatic stages of development in association with them before emerging as the winged imago (52). In these and other ways, aquatic plants exert an influence on recreational areas.

Aquatic plants exert an indirect influence on domestic animals of man. They, too, are subject to the vicious bites of insects which breed in association with these plants. Loss in quality and, in extreme cases, loss of the animals themselves have resulted from attacks by bloodsucking insects (44). In other cases bloodsucking insects may be vectors of animal diseases. For example, the stable fly and certain mosquitoes transmit the virus of equine encephalomyelitis to horses and mules (62). Livestock may be poisoned by drinking water from ponds with waterblooms of blue-green algae (64). Cattle and sheep are sometimes poisoned by eating the leaves of arrowgrass (*Triglochin maritima*) (68).

The control and management of aquatic plants sometimes may be handled cooperatively for simultaneous benefits to public health, fish and wildlife, and other interests (101). Real conflicts in interests do occur, but some of the problems may be resolved within practical limits by building on mutual interests and over-all objectives, (100). A noteworthy example is the current practice for promoting waterfowl food plants by setting plants of wetlands back to earlier stages of colonization (101). This may be accomplished by mechanical or herbicidal control of undesirable woody species, or by flooding followed by summertime dewatering to promote the growth of desirable marginal food plants.

II. Some Aquatic Weeds and Taxa Represented

The meaning of the term "aquatic plant" is subject to various interpretations. Aquatic plants, as considered here, are those species which normally *must* grow for at least part of their life cycle in water, either submersed or emersed. This concept, modified from Muenscher, includes some borderline species sometimes listed as plants of marshes and bogs (67). It excludes water-tolerant woody plants, such as bald cypress, tupelo gum, buttonbush, and black willow (34). These species grow well when stranded, and bald cypress at least thrives on terrestrial sites where planted as an ornamental. Some water-tolerant woody species are economically important weeds associated with the aquatic environment.

TABLE I. Some Common Aquatic Weeds (Angiosperms) of the United States[a]

Latin family name	Common family name	Examples
Saururaceae	Lizardtail	*Saururus cernuus*
Typhaceae	Cattail	*Typha angustifolia* *Typha latifolia*
Potamogetonaceae	Pondweed	*Potamogeton crispus*[b] *Potamogeton diversifolius* *Potamogeton nodosus* *Potamogeton spp.*
Najadaceae	Naiad	*Najas guadalupensis* *Najas minor*[b] *Zannichellia palustris*
Juncaginaceae	Arrowgrass	*Triglochin maritima*
Alismaceae	Water plantain	*Echinodorus radicans* *Sagittaria latifolia*
Hydrocharitaceae	Frogbit	*Anacharis canadensis* *Anacharis densa*[b] *Limnobium spongia* *Vallisneria americana*
Gramineae	Grass	*Phragmites communis* *Zizaniopsis miliacea*
Cyperaceae	Sedge	*Eleocharis quadrangulata* *Scirpus validus*
Araceae	Arum	*Peltandra virginica* *Pistia stratiotes*
Lemnaceae	Duckweed	*Lemna minor* *Spirodela polyrhiza*
Pontederiaceae	Pickerelweed	*Eichhornia crassipes*[b] *Heteranthera dubia* *Pontederia cordata*
Juncaceae	Rush	*Juncus effusus*
Polygonaceae	Buckwheat	*Polygonum coccineum* *Polygonum hydropiperoides*
Amaranthaceae	Amaranth	*Alternanthera philoxeroides*
Ceratophyllaceae	Hornwort	*Ceratophyllum demersum*
Nymphaceae	Waterlily	*Brasenia schreberi* *Cabomba caroliniana* *Nelumbo pentapetala* *Nuphar advena*
Cruciferae	Mustard	*Nasturtium officinale*[b]
Onagraceae	Evening primrose	*Jussiaea diffusa*
Haloragidaceae	Water milfoil	*Myriophyllum brasiliense*[b] *Myriophyllum heterophyllum*
Lentibulariaceae	Bladderwort	*Utricularia gibba*
Lythraceae	Loosestrife	*Decodon verticillatus*
Trapaceae	Waternut	*Trapa natans*[b] (water chestnut)
Primulaceae	Primrose	*Hottonia inflata*
Acanthaceae	Acanthus	*Justicia americana*

[a] Nomenclature after Muenscher (67), except *Saururus*. [b] Introduced to U. S.

Aquatic weeds are considered here to be any aggrading aquatic plants which occur where they are not desired by man. They may be either microscopic or macroscopic in size. Such plants occur in a diversity of forms and in environments ranging from running water streams to quiet waters of lakes, ponds, swamps, and other inundated areas. The four major divisions of the plant kingdom are represented among the aquatic weeds. Among the numerous species of the thallophytes are diatoms, desmids, filamentous green algae, blue-greens of waterblooms. Only a few species of the bryophytes and pteridophytes may be considered as aquatic weeds; representatives are a floating liverwort, *Ricciocarpus natans*, and the water ferns *Azolla caroliniana* and *Ceratopteris pteridoides* (25,67). The spermatophytes, or seed plants, contribute a larger number of species of macroscopic aquatic weeds than any of the other groups, all of which are angiosperms. As far as is known, there are not any truly aquatic gymnosperms, weedy or otherwise. Aquatic weeds of angiosperms are well represented by monocotyledonous and dicotyledonous plants. Important examples of aquatic weedy monocots are water hyacinth (*E. crassipes*), naiad (*Najas guadalupensis*), and cattail (*Typha latifolia*); among the dicots are such species as the water chestnut (*T. natans*), coontail (*Ceratophyllum demersum*), and lotus (*Nelumbo pentapetala*). More than twenty different plant families of angiosperms are represented by aquatic weeds in the United States (see Table I). Thus, it appears clear that aquatic weeds represent diverse taxonomic groups.

III. Introduced and Native Aquatic Weeds of the United States

As far as is known, all but seven species (67) of the more important aquatic weedy angiosperms are native to the United States. Of the introduced species, one is from Asia—water chestnut (*T. natans*); three are from Europe—watercress (*Nasturtium officinale*), naiad (*Najas minor*), and pondweed (*Potamogeton crispus*); and three are from South America—water hyacinth (*E. crassipes*), parrots-feather (*M. brasiliense*), and waterweed (*Anacharis densa*) (67). In general, the species from Europe and Asia appear in the northeastern United States and spread southward and westward. Species from South America tend to appear first in the southern United States and then spread northward, eastward, and westward. As some physiographic regions have not yet been invaded by some of these aquatic weeds, the surveillance of neighboring watersheds for the early discovery of economically important aquatic weeds, such as water hyacinth and water chestnut, and early treatment for their eradication would appear desirable. Information on the sources of aquatic weeds may be helpful in this manner.

The first record of occurrence of the water chestnut was at Collins Lake at Scotia, New York, in 1884 (66). This plant thrives in filthy water where sewage is present (52). The plant has also been found in the Mohawk River and Hudson River in New York, in the Sudbury, Concord, and Assabet Rivers in Massachusetts, in the Potomac River near Washington, D. C., and in the upper section of Chesapeake Bay (59).

Accounts of the origin, place, and date of introduction of water hyacinth into the United States differ. As best as can be determined, the plant appears to have been introduced at the Cotton Centennial Exposition in New Orleans, Louisiana, in 1884 (27,47,78). It appeared in Florida in about 1890, and by 1920 it was reported from California (45). Since its introduction it has become one of the most important aquatic weeds in the southeastern United States. In 1935 it was found near Harris Station, Limestone County, in North Alabama by Fairer and removed in 1935 and 1936 without further recurrence (24).

The history of the introduction of the other species, with the possible exception of *N. minor*, apparently is not as well known as the above and will not be commented upon. The naiad (*N. minor*) is known only from the Hudson River, Lake Ontario, and the Tennessee Valley (67).

IV. Nature of Aquatic Weeds

Aquatic plants show a diversity of form and structure. The lower plants, which evolved in water, usually are simple cells or groups of cells without organization into roots, stems, and leaves. On the other hand, the higher vascular plants usually have vegetative bodies consisting of the above organs. These higher plants apparently have reinvaded the aquatic environment and have developed from progenitors which lived on land (102). The presence of vestigial stomata and other atrophied structures in some submersed plants lends support to this view (5). The ecological anatomy of higher aquatic plants of diverse phylogenetic origins often reveals similar structural adaptations to the uniformity of the aquatic environment and its attendant condition of poor aeration. Aquatic plants are adapted to grow under conditions of low oxygen tensions such as occur in water or in soils covered by or saturated with water. Poor aeration excludes most terrestrial species from the aquatic environment. Terrestrial plants will grow in water where oxygen is supplied through special aeration methods (97). The one feature that is common to most of the higher aquatic plants is the presence of spongy or aerated tissue (20). Sponginess is due to the presence of air chambers or air passageways and to a special type of tissue known as aerenchyma. The air chambers or lacunae are intercellular spaces and these, when elongated, are known as canals. These intercellular spaces apparently form an internal system for gas retention or conduction within the vegetative bodies (22). Blade-bearing detached petioles of lotus commonly bubble gases in sunlight. Air spaces are formed by separation of cells and by the disintegration of cells (22).

The roots of aquatic plants are variously modified. In some species they are absent in both the seedlings and the mature plant. Species of this nature are coontail (*C. demersum*) and bladderwort (*Utricularia gibba*) (67). Other species lack a primary root on germination of the seed but develop roots at nodes of the stems. Representatives of this type are water chestnut (*T. natans*) and lotus (*N. pentapetala*). In the duckweed (*Lemna*) the root system is reduced to a single axis bearing a root pocket but lacking root hairs. In the greater duckweed (*Spirodela*) several reduced roots are present (67). A number of species of aquatic plants bear roots but lack root hairs. In cattail (*Typha*) the abundance of root hairs varies with the degree of aeration (97).

The stems and leaves of aquatic plants show different degrees of adaptation to the aquatic environment. Submersed species exhibit loss of supporting tissue, reduction in conducting tissue, loss of cutin, and chloroplast-bearing epidermal cells (22). A submersed aquatic may be likened to a thallus growing in a nutrient solution. Emersed species commonly have well-developed mechanical tissue, some conducting tissue, cutin, and chloroplasts mainly inside the epidermal layer. Stems of emersed plants may be entirely underground as in *Nelumbo,* both underground and erect as in *Saururus,* or stoloniferous and erect as in *Justicia americana.* Horizontal floating stems occur in other plants, such as water hyacinth and water primrose (*J. diffusa*). Aerenchyma forms sheathing spongy cylinders of stems of plants such as swamp loosestrife (*Decodon verticillatus*) and water primrose. This special type of tissue arises from a phellogen layer, being either epidermal or cortical in origin (22). Aerenchyma of stems and roots adds buoyancy to a floating-mat species such as water primrose.

The leaves of aquatic plants also take various forms. In submersed species the leaves may be strap-like as in *Elodea* or much dissected as in *Cabomba caroliniana.* The plant-water interface is increased by dissection of leaves, which favors interchange of gases and uptake of nutrients. In a plant such as *Proserpinaca palustris* the lower submersed leaves are dissected, but the aerial leaves are flat blades. The aerial leaves of other emersed species may be floating or erect, borne on aerial stems, or greatly reduced as in *Scirpus.* The leaves of some species may be modified into traps for capturing aquatic organisms, as in *Utricularia vulgaris* and *Aldrovanda vesiculosa* (54).

Aquatic plants may reproduce either by seed, vegetatively, or by both methods. The longevity of seed ranges from one to several years, as in *T. natans,* to a hundred years and more, as in *Nelumbo* (8,67). Seed production in the United States is not known for *A. philoxeroides,* and reproduction appears to be entirely by vegetative means. In some species vegetative propagation may also occur by winter buds, as in *Ceratophyllum* and *Potamogeton* (67). The density of winter fronds of *Spirodela* is greater than that of water, and these plants may overwinter on the bottom and return to the surface the following spring (5). Stolons, rhizomes, tubers, stems, and even leaves (as in *Rorippa aquatica*) serve as vegetative reproductive and overwintering structures. In *Nymphoides aquaticum* flowers and roots arise from the outer portion of the petiole.

V. Growth Form in Relation to Control Procedures

As a general rule, important aquatic weeds are colonial or gregarious species with high colonization potentials. Most are perennials, but some, such as *T. natans,* are annuals. Even though diverse taxonomic groups are represented, several growth forms have developed in response to similar environments. Since the ecological growth form influences the practical methods for control, it offers a useful basis for classifying aquatic weeds. The suggested classification which follows is modified from that of Hess (38). It does not have sharp boundaries owing to the diversity in species and even to different growth forms within a species.

- Aquatic plants
  - Submersed
    - Suspended. *Examples:* phytoplankton; duckweed (*Lemna trisulca*)
    - Anchored. *Examples:* coontail (*Ceratophyllum demersum*); muskgrass (*Chara*)
  - Emersed
    - Free-floating. *Examples:* water hyacinth (*Eichhornia crassipes*); water lettuce (*Pistia stratiotes*)
    - Anchored
      - Floating-leaf. *Examples:* lotus (*Nelumbo pentapetala*); cowlily (*Nuphar advena*)
      - Floating-mat. *Examples:* alligator weed (*Alternanthera philoxeroides*); water primrose (*Jussiaea diffusa*)
      - Erect leafy. *Examples:* water willow weed (*Justicia americana*)
      - Erect naked. *Examples:* softrush (*Juncus effusus*); cattail (*Typha latifolia*)
- Water-tolerant woody species. *Examples:* black willow (*Salix nigra*); buttonbush (*Cephalanthus occidentalis*)

A. AQUATIC PLANTS—SUBMERSED

1. Suspended Species

Suspended species are those submersed aquatic plants which are surrounded on all sides by and drift about in water. These plants must secure oxygen, carbon dioxide, and mineral nutrients from the water. Even light for photosynthesis must pass through water. For practical purposes, the algae are the important members of the group (74,84).

The possible control procedures for suspended plants are limited not only by location but also by the small size, abundance, and high and rapid reproductive capacity of the plants themselves. Two control methods are used: application of toxic chemicals and exclusion of light. Copper sulfate and chlorine are two chemicals which have been used widely and safely for the control of algae, including those in domestic water supplies (99). Many other chemicals might be used as algicides. Some other algicides of promise are reported in the literature (73).

Chemical control requires the distribution of the toxicant throughout the water so that the minimum lethal dose is in contact with the organisms for certain retention periods. If application of the chemical is properly timed, the winds will promote mixing, and advantage may also be taken of convection currents (99). For example, if the algae are close to the surface, the chemical should be applied early in the day; if they are widely distributed vertically, it should be applied toward night, since it will tend to sink as the surface strata cool. Sometimes the algae move through vertical cycles in pools because of the increase and decrease of entrapped bubbles of gaseous oxygen during alternating periods. These forms may be treated more effectively when near the surface than when far below.

In some situations algal growths affecting domestic water supplies have been eliminated as sources of tastes and odors by covering the supply reservoir with a light-tight canopy to exclude light. As a rule, all finished water basins or clear wells should be covered. Sunlight may also be excluded by the addition of activated carbon to the reservoir (40), which produces a blackout.

2. Anchored Species

Anchored submersed aquatics are those species growing beneath the water surface and attached to a substratum. Their growth zone, once established, is well defined and can even be mapped. Unlike the suspended species, they do not as a rule drift about at the mercy of the currents. However, noteworthy exceptions to this are sometimes encountered in the genera *Ceratophyllum* and *Utricularia* where the plants break loose from the substratum. Submersed anchored species include algae and angiosperms. Although the algae may usually be controlled in the manner indicated under suspended species, associated anchored vascular plants, for unknown reasons, commonly survive the same treatment.

These vascular plants show various adaptations to their aquatic environments. A knowledge of their ecological anatomy may be helpful in understanding possible control methods, which are related to growth forms. Adaptations include reductions in protective, supporting, and conducting tissues. The epidermis lacks cutin, the epidermal cells contain chloroplasts, the xylem is atrophied, the phloem is reduced but still well developed, and root hairs are usually lacking or the roots are reduced to holdfasts or even absent (20,22,67). The plants absorb nutrients, gases, and water directly from the aquatic environment in which they are submersed and are supported by the buoyant effect of the water and by gases entrapped in their air chambers. The air chambers accumulate excesses of oxygen in the daytime and of carbon dioxide at night. These gases within the vegetative body are used in the complementary processes of respiration and photosynthesis (20).

Because these plants are macroscopic and grow in specific locations, they can be controlled by hand removal or removal by mechanized equipment where applicable. The fact that the epidermis lacks cutin permits the effective control of these types of plants by periodic dewatering and exposing the stranded plants to the hot rays of the sun which promote rapid drying and death. The application of chemicals for the control of these types of plants where confined is often practical. The minimum lethal dose may be applied throughout the water or toxic formulations with specific gravity greater than water may be introduced as liquids by special equipment to the colonized strata. These plants may also be controlled in some instances by the application of pellets or granular herbicides which are heavier than water (28). In general, on the basis of plant structure, translocated herbicides would not be expected to give favorable results with these types of plants unless the toxic agent was present throughout the water surrounding the plant. This appears to be sound in practice, since short or immature plants close to the source of the herbicide on the bottom are controlled more readily than taller and more mature specimens (4a).

B. AQUATIC PLANTS—EMERSED

1. Free-Floating

Emersed free-floating aquatics are those unattached species that live partly above and partly below the air-water interface. Because the lakeward colonization, of important species at least, is not limited by increasing water depth, these types of plants often cover extensive areas of relatively quiet bodies of water. For some species the potential area of colonization appears to be limited only by the extent of the surface area of the body of water itself. Plants in this group range in size from the largest of these—water hyacinth (*E. crassipes*) —down to the duckweeds (*Lemna minor* and *Spirodela polyrhiza*), and finally to the smallest flowering plants known (*Wolffia* spp.). Parenthetically, this tiny flowering plant is about the size of a pinhead and lacks roots (67). From the point of view of control, these types of plants all present a surface above the water that is relatively easy to reach with a toxic spray applied by aircraft or by other means in geographically readily accessible areas. Because of the close proximity of the above and below water portions of the plants, translocated herbicides have relatively short distances of downward transport. The floating habit of water hyacinth has led to the construction of barrier booms across bayous to prevent its migration to new areas (91). Crushers, conveyors, and other methods of mechanical removal have also been employed in the control of free-floating plants (78). Pulling of these plants must be followed by removal to higher ground, by crushing and cutting before returning to the water, or by piling on themselves in water, in order to obtain control by mechanical means.

2. Anchored

Floating-Leaf. Anchored floating-leaf aquatics are those species rooted in the substratum and with relatively long petioles connecting the aerial but floating leaf blades to the rhizomes, stolons, or other types of submersed stems. These plants usually grow in dense colonies in water ranging from about 1 to 8 feet in depth. Representatives of this group include such plants as lotus (*N. pentapetala*), cowlily (*Nuphar advena*), watershield (*Brasenia schreberi*), and waterlily (*Nymphaea odorata*) (33). Some species have leaves elevated above the water surface in addition to the floating leaves. The plants enumerated are all perennials, and colonies persist from year to year. They overwinter in various ways, but all store food in their persistent horizontal stems. The repetitive cutting or removal of successive sets of leaf blades tends to deplete the food reserves; the plants use up food reserves in sending forth new leaves, and further synthesis and storage of foods are retarded by detaching the new leaves. The application of this principle has received wide application in the control of these types of plants. Since leaves do not generally serve as vegetative reproductive structures, their removal from the water following cutting is not necessary. Hand removal, weighted underwater saws, and shallow draft underwater weedcutters have been used to sever the easily cut petioles of these types of plants. These species also have large leaf surfaces more or less horizontally disposed above the waterline where they can be readily reached by toxic sprays dispersed by aircraft. Sprays may also be effectively dispersed from boats and by other means in readily accessible areas.

Floating-Mat. These aquatics are those species commonly rooted along the shoreline with horizontal, leafy, floating stems growing lakeward. The lakeward extension of these colonies in areas with subfreezing temperatures is usually limited to one season of growth, the plants renewing growth from perennating structures near the water edge from year to year. On relatively steep shorelines, colonies of these plants form well-developed bands of vegetation extending out over the water surface independent of water depth. Shallow, flat, and sheltered areas periodically dewatered favor establishment of these types of plants, which may cover extensive areas. The floating stems may be hollow, as in alligator weed (*A. philoxeroides*), or they may be well supplied with cottony cylinders of aerenchyma, as in the water primrose (*J. diffusa*) (76). Some of the adventitious roots of water primrose are also cottony owing to aerenchyma. These features give buoyancy to the colonies.

Following strandage in favorable sites either along the shoreline or on drift and flotage, node-bearing fragments of these plants give rise to new colonies. This means of vegetative reproduction eliminates mowing, cutting, chopping, or crushing of these plants as desirable methods of control; such methods tend to spread the plant to new areas by setting these vegetative reproductive parts free in the water for transport to new sites. Good control of the aboveground parts of alligator weed can be obtained. Along relatively steep shorelines, this may be accomplished by spraying the landward portion of the mat, by rolling the water portion of the mat back on land during a period of low water levels, and by spraying with herbicides. Stolons of alligator weed are usually buried under old mats of these plants, where they generally survive herbicidal treatments on colloidal soils. On rocky sites, underground portions may be effectively controlled by spraying when the plants are dewatered. Established stands of water primrose can be controlled by the use of herbicidal sprays even while the plants are in water. In areas subjected to subfreezing temperatures, alligator weed and water primrose may be attenuated and sometimes even controlled if the plants are dewatered during the subfreezing periods. Freezing of colonies partly on land and partly on water sets free the outer protected portions of colonies in water, which may then be water borne to new areas and further spread the species.

Erect Leafy. Anchored, emersed, erect leafy aquatics are those species rooted to the substratum and commonly with horizontal stems from which arise vertical stems bearing an aerial leafy canopy. The horizontal stems may be either stolons or rhizomes. These types of plants commonly grow in water from about 1 to 4 feet in depth but also may occur in shallower or even deeper water. Examples of this group include water willow weed (*J. americana*) and lizardtail (*Saururus cernuus*) (29,75). These plants are perennials, and the colonies persist from year to year by overwintering rootstock. Detached stem fragments from these plants float and develop roots and shoots from the nodes. These fragments serve as vegetative reproductive structures for starting new colonies on strandage at favorable sites. For this and other reasons, mechanical measures, such as repetitive cutting, are not considered practical, even though they may yield a degree of control at the treated site. Wintertime dewatering with exposure of the rootstock to subfreezing temperatures at a latitude of about 35° N has not given any practical control or even any apparent attenuation to exposed colonies. For these types of plants herbicidal methods of control, where applicable, would be the method of choice. The presence of a leafy canopy presents a relatively large surface for the reception of foliage sprays.

Erect Naked. Erect, naked, emersed aquatics are considered to be those species rooted in the substratum and usually lacking well-defined leaves; if leaves are present, they are linear and erect. Examples of this group include bluestem (*Scirpus validus*), cattail (*T. latifolia*), softrush (*Juncus effusus*), four-angled spikerush (*Eleocharis quadrangulata*), and scouring rush (*Equisetum*). Plants in this group are perennial colonial species with horizontal rootstock. These species sometimes grow in the inner and shallower portion of the littoral zone, and they are sometimes referred to as amphibious plants. They show certain adaptations to the aquatic environment associated with the conditions of poor aeration of the soils in which they grow. Their ecological anatomy shows structures that permit the passage of gases from the atmosphere to the underground rootstock and other submersed parts. These structures include air chambers bounded by perforated septa through which gases diffuse from the atmosphere to the roots and vice versa (97). Entrance and exit of the gases are via the stomata. The stomata of some of these species are open continuously, day and night, as in bluestem (*S. validus*), scouring rush (*Equisetum hiemale*), and cattail (*T. latifolia*) (55,97). This condition favors the diffusion of gases in and out of the underground parts to provide oxygen for respiration and for the removal of carbon dioxide. This dependence of the underground structures on aerial portions for aeration has been used to advantage in the practical control of cattail. A common practice used for control consists of plowing the cattail colony near the water edge to break the rhizome or air conduit and cutting the erect portions of the plants below the water surface (97). This method, or merely cutting and flooding where the entire colony is below the new waterline, may give good control. In some situations two cuttings in one growing season may be required (90). In addition to cutting and flooding, some of these species can be readily controlled by the use of herbicides applied even as aqueous solutions. The aerial portions of these types of plants present waxy, almost vertical surfaces of relatively small area for interception of sprays (23). The angiosperms involved, softrush and four-angled rush, are monocots, and accumulation of runoff droplets near their basal growing areas might account in part for their control with aqueous solutions of 2,4-D. The presence of continually open stomata in species like bluestem suggests that sprays of low surface tension or fume-producing herbicides might be used to advantage. When wide open, stomates are penetrated by such solvents as alcohol, xylol, and benzol; and the lower the surface tension, the smaller the stomatal opening penetrated (19).

C. WATER-TOLERANT WOODY SPECIES

This group includes trees, shrubs, and vines which usually survive continuous flooding to a depth of 1 or more feet for two or more consecutive growing seasons (77). Common examples of these woody weeds include black willow (*S. nigra*), buttonbush (*C. occidentalis*), and trumpet vine (*Campsis radicans*). These plants do not show special anatomical modifications, such as the development of aerenchyma or air passageways, as noted for the herbaceous aquatics. Sometimes adventitious roots are produced on the stems of black willow and buttonbush during flooding in the growing season. They may form huge "beards" even in the tops of deeply inundated black willows. These plants tolerate poor aeration and grow well on periodically flooded or wet soils. Water-tolerant trees and shrubs are considered to be poor competitors and colonize low sites where water excludes more aggressive mesic species.

Black willow, buttonbush, and trumpet vine sprout from stumps or stubble following initial cutting any month during the frostless season. Annual repetitive cutting of the coppice from stumps or stubble gives no practical help toward the elimination of these plants. If the coppice projects above the water surface, survival may be expected for at least several seasons even in relatively deep water. Thus, the coppice of these species is also water tolerant. If the plants are cut close to the ground level in the dormant season and flooded before growth starts in the spring, the stumps or stubble will not sprout as long as the cut surface is continually flooded. Following such flooding black willow succumbs by early summer. Stumps and stubble of buttonbush survive continuous flooding for at least several growing seasons. These structures can be maintained in a dormant condition by cutting and flooding, but on dewatering in the frostless season growth resumes and buttonbush populations persist. Trumpet vine appears to occupy an intermediate position in response to this type of cutting and flooding. Old stumps of tupelo gum produce coppice, but this has been observed to die off without any special treatment. Coppice from young tupelo gum usually persists. In general, it appears that the sprouting potential of water-tolerant trees decreases with an increase in age.

Species in this group all have aerial foliage, and some may be controlled by herbicides applied as foliage sprays (32). Stump sprays, bark sprays, and girdling, frilling, and spraying may all be used successfully on black willow (12,60,77). Buttonbush is highly resistant to herbicidal treatments, and there remains a need for a practical method for its control. Salt cedar (*Tamarix pentandra*) is an important woody weed of the Southwest and also is difficult to control (60).

VI. Nature of Compounds for Penetration of Cuticle and Cells

The polarity of compounds has important relationships to herbicides and their entrance through the leaf cuticle and roots, as pointed out by Crafts (13). Compounds may be either polar or nonpolar. Most inorganic compounds are polar and most organic compounds are nonpolar or relatively so (53). Polar compounds have molecules with electropositive and electronegative regions and tend to form ions (63). Sodium chloride and other similar inorganic salts or electrolytes are examples of strongly polar compounds. In organic compounds the electrical charges may be on only a portion of the molecule, owing to the presence of polar groups. Examples of such polar groups include —OH, —COOH, —NH_2, —CHO, and unsaturated groups (19). For example, in acetic acid the polar nature is due to the —COOH group, the —CH_3 group being electrically inert. Water is a polar compound. Polar substances generally dissolve more readily in polar solvents, such as water, than in relatively nonpolar solvents, such as benzene or other hydrocarbons. Nonpolar compounds are essentially electrically inert and do not tend to form ions (19). Examples of these include saturated aliphatic and saturated ring or naphthenic compounds such as hexane and cyclohexane (14). Other oily compounds such as benzene and other products from coal tar and petroleum, are relatively nonpolar. Nonpolar groups include —CH_3, —C_2H_5, and —C_6H_5 (19). The addition of polar groups such as —OH as in the alcohols results in a polar compound. The longer the carbon chain in proportion to the number of —OH groups, the less is the polarity of the molecule. Nonpolar substances, or those relatively so, dissolve more readily in organic substances such as cyclohexane, benzene, and other hydrocarbons than in water. The cuticle of leaves is nonpolar or relatively so. Therefore, relatively nonpolar toxicants and carriers would be expected to enter through the leaf cuticle more readily than polar compounds. Polar and nonpolar pathways across the cuticle have been suggested; the complexity of this problem has been reviewed by Currier and Dybing (18). Nonpolar compounds enter cell membranes more readily than most polar compounds. Lowering the polarity of alcohol by increasing the number of nonpolar groups (CH_3OH to C_2H_5OH) results in the more rapid entrance; ethyl alcohol enters faster than methyl alcohol. Increase in the number of —OH groups increases polarity and decreases the rate of entrance (19). Water, a polar compound, enters cells readily, but most polar compounds or their ions enter cells with difficulty (19). This also indicates that nonpolar compounds, or those relatively so, would be favored for herbicides for entering the cuticle and the cells. In practice, polar compounds are favored as herbicides for absorption by roots from soils (13).

The polarity concept appears to offer a sound basis in relation to herbicides and their penetration of the cuticle and cell wall and entrance into cells. This concept serves well as a guideline in attempting to solve practical plant control problems. Some exceptions to the general rules of entrance of substances in relation to polarity should be noted. Polar compounds, such as sulfuric acid and sodium arsenite, penetrate leaf cuticles, but this is by their corrosive action (13). Exceptions to the general rule apparently also occur with the hormone type of herbicide, such as 2,4-D, applied as foliage sprays. Salt and ester derivatives enter through the leaf surface and penetrate cells, as indicated by their translocation in the phloem and accumulation in meristems. Some species of plants may be controlled as readily with salts as with esters of 2,4-D; for others esters are favored. Some plants may be controlled with these 2,4-D herbicides applied as foliage sprays with application rates of 1 lb. per acre and even less on an acid equivalent basis. Considering the minute quantities of these toxicants required, it is possible that the lethal dose may enter through cracks in the cuticle (13). With small quantities of toxicant necessary, the relative rate of penetration into the cells of leaves does not appear to be of limiting importance. In general, the esters are recognized as being more effective as foliage sprays for the control of some species, or even for some growth stages within a species difficult to control with the salts. For root uptake of the hormone type of herbicides from soils, the salts are favored, but esters may also be effective by hydrolyzing to the acid form which enters roots from the soil solution (13). Crafts gives the following helpful generalization for absorption of various herbicides: "for penetration of cuticle and absorption by foliage, nonpolar compounds should be used; for absorption by roots, polar compounds" (13). The mode of action of various herbicides has been reviewed by Crafts (15). Four modes of movement of herbicides in plants are given by Crafts and Foy (17) from studies with tagged compounds.

VII. Nature of Leaf Surface

The surface of leaves of emersed aquatics is highly impervious to water because of the presence of a waxy, noncellular covering or cuticle. This layer is almost continuous over the leaf surface, being interrupted at the stomatal pores and by cracks in its surface. The cuticle appears to be secreted by the epidermal cells. The walls of the epidermal cells may also be infiltrated by cutin. Cuticularization and cutinization both tend to prevent the loss of water from leaves and from other structures (22). The presence of these waxy substances also prevents water from readily wetting leaf surfaces. Sometimes epidermal projections, as on the leaves of lotus, or hairs, as on the leaves of *Pistia*, also contribute to the shedding of water by these plants (33). The general nature of the leaf surface has implications in relation to the use of foliage sprays. The cutin is electronegative in its charge, and water, polar solutes, and particularly anions do not readily penetrate it (79).

The chemical nature of the waxy substances in lotus leaves is of interest and has been studied by Horner (41). The total amount of wax in the leaves was 1% of their total weight. Of this not more than half was a water-resisting dihydric alcohol ($C_{28}H_{58}O_2$) with a melting point of 105–106°C. The paraffin fraction consisted chiefly of *n*-nonacosane ($C_{29}H_{60}$) with small amounts of higher and lower homologues. Alcohols and paraffins in plant waxes are solids at ordinary temperatures (9). The hydrocarbons included in the wax component have odd numbers of carbon atoms ranging in different plants from C_{25} toC_{37} (9). The acid fraction from lotus appeared to consist of a mixture of hydroxy acids and normal fatty acids. Most leaf waxes consist mainly of primary alcohols with small amounts of fatty acids and paraffins (41). These are predominantly nonpolar compounds. In accordance with Crafts' theory, nonpolar sprays would normally enter best through surfaces containing these compounds.

VIII. Methods of Control

A. CHEMICAL

Many kinds of algae may be controlled through the use of copper sulfate, chlorine, and other algicides (99). In general, algae are extremely sensitive to copper. There is sufficient copper in distilled water prepared in a copper still to produce toxicity to *Spirogyra* in the absence of antagonizing ions (19). Copper sulfate has been widely used as an algicide since 1904 with effective results. Applications for algal control should be started before the species involved reach the nuisance level. A knowledge of local conditions regarding seasonal algal populations and microscopic counts are helpful in determining the time to begin treatment. The amounts of copper sulfate required to kill various species of algae differ, and shifts in algal populations may result (74). Control was obtained of some blue-green algae with 0.12–0.5 p.p.m., of some green algae with 0.05–10 p.p.m., and of some diatoms with 0.07–0.5 p.p.m. of copper sulfate (99). Some species of algae may be controlled with 1 p.p.m. of copper sulfate or less. Copper in treated domestic water supplies, in accordance with the U. S. Public Health Service drinking water standards, should not exceed 3.0 p.p.m. (92). This is equivalent to 7.5 p.p.m. of copper sulfate. Fish toxicity begins at 0.14 p.p.m. for trout and at 2.10 p.p.m. copper sulfate for bass (99). Hirsch, referring to copper sulfate, states, "Due to the precipitation and loss of copper from alkaline waters, a higher dose up to about 2.5 p.p.m. and over usually is safe for fish when applied under proper control" (40).

Copper sulfate may be applied by boat, by dragging burlap sacks of the material through the area to be treated. The path taken may be either crisscross triangles for small reservoirs or checkerboard fashion with two series of parallel swaths, these at right angles to one another. Treatment swaths should be about 25–100 feet apart, depending upon the reservoir size, depth, and dosage (40,99). Copper sulfate may also be applied as a solution at the stern of the boat and use made of the propeller for mixing (40). It may also be applied as a spray or as a finely powdered dust on the water surface. The desired amount of copper sulfate can be approximated from the known volume of water, 8.3 lb. of copper sulfate per million gallons of water equaling 1 p.p.m. of copper sulfate. The volume of water over 1 acre with a uniform depth of 3 feet equals 980,100, or approximately 1 million, gal. An alga, such as *Spirogyra*, with a lethal concentration of 0.12 p.p.m., would require only approximately 1 lb. of copper sulfate per million gallons of water.

Chlorine is also used as an algicide in water treatment plants, in swimming pools, and in other situations. Chlorine is commonly added as liquid chlorine, as a solution of sodium hypochlorite, or as a powder, calcium hypochlorite (3). The prechlorination treatment of raw water at the intake previous to filtration sometimes supplants copper sulfate treatments of the supply reservoir. The prechlorination dose may range from 0.5 to 5.0 p.p.m. or more, depending upon the organic and mineral content of the water (3).

Sodium arsenite, in some situations, may be of practical use as an algicide. It has been used in fish ponds for the control of algae such as *Pithophora* and in other bodies of water (51). Because of its arsenical nature, it should, if used, be respected, as people, cattle, and other organisms may be poisoned or even killed. It can be used safely in ponds without damaging fish and without impairing their fitness for human consumption (86). Sodium arsenite should never be used around drinking water supplies or in ponds with overflow drainage to drinking water supply intakes.

Many compounds offer promise for the control of algae, including 3-(*p*-chlorophenyl)-1,1-dimethylurea and 2,4-dichloro-1,4-naphthoquinone (4,58,73).

Submersed vascular plants may be controlled by chemicals, especially in confined situations such as ponds and ditches. In quiet water, sodium arsenite applied at a concentration of 4 p.p.m. may be effective for the control of plants such as *Najas* and *Elodea* (51). In local areas of larger lakes lateral diffusion may require concentrations of 6–10 p.p.m. sodium arsenite for controlling submersed species. Fish are not killed below 11–12 p.p.m. sodium arsenite, and the above herbicidal dosages are below this threshold (46). Treated areas should not be used for swimming, boating, or by farm animals for a 3 day period following treatment (46). For the control of submersed species in ditches emulsifiable aromatic solvents such as methylated benzenes (xylene) and some other unsaturated hydrocarbons may be used satisfactorily (11,85). For example, gasoline and polychlorobenzene mixtures gave good control of *Najas* and *Ceratophyllum* (46). These types of treatments kill not only submersed plants but also fish and other organisms (85). In California the continuous solution feeding of copper sulfate in small concentrations to water supply reservoirs has given effective control of submersed vascular plants such as pondweeds (98). Pellets containing 2,4-D are offering promise for control of submersed species. Acrolein has herbicidal properties and can be used in canals for the control of submersed plants and also as a molluscicide (95). The chemical structure and mode of action of many herbicides have been reviewed by Crafts (16). The mode of action of phytotoxic oils has been reviewed by Van Overbeek and Blondeau (94).

A number of species of emersed aquatic plants may be controlled effectively by herbicides. Since the control effectiveness of herbicides varies with species, some examples of herbicide and species combinations favorable to control will be given. The availability of highly effective herbicides has resulted in the control of some species to a degree not attainable by the more cumbersome mechanical measures.

The hormone type of herbicide, 2,4-dichlorophenoxyacetic acid and its salt and ester derivatives, is effective in the control of many species. The salt forms are water soluble, and the ester forms are oil soluble. These are used for the preparation of aqueous sprays, oil sprays, and emulsified sprays. The acid form has low solubilities in aliphatic oils and in water. Aqueous sprays of the acid form may be prepared by the addition of polyethylene glycol (65). Application rates are usually expressed in terms of the acid equivalent of the molecule to which phytotoxicity is related. The esters are more phytotoxic per pound of acid equivalent than the salts (46). The amine salt is more phytotoxic on this basis than other salts (46). The salts are practically nonvolatile and, where effective on the weed, should be favored for use near susceptible crop plants (46). The compound 4-chloro-2-methylphenoxyacetic acid also may be used as an effective herbicide (83).

Examples of emersed aquatic plants which may be effectively controlled with 2,4-D include the following representatives of different growth forms. Among the free-floating species, the water hyacinth (*E. crassipes*) can be controlled effectively (21,56,60,105). In the floating-leaf group, American lotus (*N. pentapetala*) also responds favorably to 2,4-D treatment (30–32,46). In the attached floating-mat group, water primrose (*J. diffusa*) may be controlled with these types of sprays (32,60). The attached erect leafy water willow weed (*J. americana*) also responds favorably to these sprays (32). Erect naked plants, such as softrush (*J. effusus*) and squarestem spikerush (*E. quadrangulata*), also may be controlled by this means (32). The water-tolerant black willow (*S. nigra*) and swamp loosestrife (*D. verticillatus*) also are relatively easy to control with 2,4-D (32,60). From the above it appears clear that some monocots and dicots can be controlled effectively with 2,4-D. The volume of spray used is not considered important in relation to toxicity to herbaceous weeds as long as the minimum lethal dose is distributed evenly over the plants. Atomization of low volume sprays increases drift possibilities, and coarser higher volume sprays would be favored for this reason. For woody species, in general, higher volume sprays appear preferable.

Examples of emersed species for which other herbicides than 2,4-D are favored for control are given below. Cattail (*T. latifolia*) may be controlled with 3-amino-1,2,4-triazole (ATA) and 2,2-dichloropropionic acid (dalapon) (7,60,90). For the control of phragmites (*Phragmites communis*), dalapon, ATA, and a mixture of these two herbicides may be used effectively (60). Giant cutgrass (*Zizaniopsis miliacea*) is difficult to kill, and there is a need for an economical chemical method for its practical control (60). Giant cutgrass may be controlled with relatively high rates of application of dalapon, ammonium sulfamate, polybor-chlorate, and other compounds (60). In Texas excellent control of giant cutgrass was obtained on test plots with 20 lb. per acre of dalapon (26a). For the control of some water-tolerant woody species, mixtures of 2,4-D and 2,4,5-T, ammonium sulfate, and urea compounds may be useful (60). Details of control procedures, treatment rates, and timing of herbicidal applications are given for a number of herbaceous and woody species by Martin, Erickson, and Steenis (60). Herbicides, their properties, and sources are given in publications (16,26,70).

B. MECHANICAL

Mechanical methods of control include the various operations dealing with the cutting and removal of plants. These operations may be performed with hand tools or with mechanized equipment, such as circular watersaws, dredges, underwater mowers, and water rakes. Some plants, such as lotus, cowlily, and parrots-feather, may be controlled in this manner, but the procedures are difficult and costly compared to newer methods. Cutting of water hyacinth with circular saws has been fairly effective (78). Bank deposition of water hyacinth by means of conveyors has been used but has proved too costly because the wet weight of these plants approximates 150 tons per acre (78). Flame throwers have been ineffective against water hyacinth (27). Dynamite sometimes is used to open up silted in ditches, and this procedure simultaneously clears ditches of weeds. Mechanical cutting followed by the use of herbicides or by flooding may be helpful in the control of littoral plants.

C. PHYSICAL

1. Water Level Management

The manipulation of water levels may be used for the management and control of littoral plants. In order to regulate the water levels, control gates, drains, or other special devices are installed either in the design period of a reservoir or later. Water level management may be used to "telescope," or lessen, the lakeward extent of littoral vegetation. In some situations, depending on species, topography, nature of the bottom, and other factors of the environment, water level management may be ineffective. The effectiveness of different water schedules may vary from elimination of the littoral plants to virtually no narrowing of the vegetated zone.

Water levels exert an influence on the establishment and survival of plants in contour bands (35). Many emersed species require the strandage of seed and vegetative reproductive structures for establishment. Each anchored emersed species also has a local limiting water depth beyond which it does not colonize or survive. Establishment and survival also are impeded by the poor aeration of the aquatic environment.

The concept of using water for the management and control of littoral plants has found practical application in malaria control, in fisheries and waterfowl management, and in agriculture (34,60,79). This concept is considered important where applicable because (1) it is highly effective and economical, (2) plants along almost every foot of shoreline are influenced, whether 1 mile or 10,000 or more miles of shoreline are involved, and (3) while operative, it functions day and night as either a preventive or corrective measure. In some instances, water, properly manipulated, is the most effective and economical agent for the control and management of most littoral plants.

An over-all annual water schedule may be devised in accordance with rainfall, runoff, and evaporation cycles and within limitations of primary uses of the pool. The water level may be maintained at a relatively constant elevation, or it may be either raised or lowered. These three conditions give rise to the descriptive terms of constant pool elevation, filling, recession, and cyclical fluctuation (the alternate lowering and raising of the water levels). The water may sometimes be raised above its normal top summer pool elevation into a flood surcharge zone. A number of different combinations of the above water changes may be incorporated into annual schedules. Recession and cyclical fluctuation may be varied by amplitude and frequency, and, in some cases, seasonal recession may be incorporated with cyclical fluctuation. Reservoirs filled annually in the spring and with wide straight line recession curves may be considered to have a water schedule consisting of one annual cyclical fluctuation.

The manner in which water levels may influence littoral vegetation may be illustrated by several examples. The seeds of black willow are wind and water borne and short-lived, and they require strandage for establishment. Holding the water levels high during the period of seed dissemination of black willow will prevent its establishment in a lakeward direction. Conversely, lowering the water levels during its fruiting period will permit its establishment over extensive areas. Other water-tolerant woody species, such as buttonbush, tupelo gum, green ash, and bald cypress, require dewatering of seeds or fruits for their establishment. Reproductive structures of aquatic plants commonly float. For some important species reproductive structures usually do not strike root in the soil until they are transported to shallow areas near the existing water edge or until stranded. Examples of these include seedlings of water willow weed, softrush, cattail, and nodal fragments of alligator weed. Even in lotus, a deep water aquatic, the seedlings are known to strike root only in shallow water. Lotus seedlings become established where the water depth is less than the length of the petioles of the small peltate leaves which emerge from the attached fruit. In general, it appears that perennial emersed anchored aquatics require dewatering or shallow water for establishment. Following establishment, colonies migrate vegetatively in a lakeward direction. Most of these emersed species can tolerate, for relatively long periods, water depths greater than those in which colonization occurs. Raising the water on established colonies of lotus so that the water depth is greater than the petiole length results in the rising water snapping off the blades and attenuating the colonies. The ability of most emersed species to survive extended periods of flooding during a growing season has led to the control of some of these by cutting and then flooding the cut surfaces. Plants which may be readily controlled in this manner include black willow, cattail, and softrush. Buttonbush so treated may resprout, even following continuous flooding for more than one growing season, when dewatering re-exposes the stumps or stubble to the atmosphere. For the control of submersed species, periodic dewatering of colonies generally gives control. Sometimes submersed species disappear without any special control efforts. Free-floating emersed species merely ride up and down with changing water levels. Water cycles of small amplitudes have no apparent effect on these and other floating-mat species. The wintertime dewatering and exposure of stranded plants of alligator weed and water primrose to subfreezing temperatures at a latitude near 35°N appear to be of real help in attenuating these species. Colonies overwintering in water are protected from subfreezing air temperatures, and floating fragments are set loose by the landward portions of colonies freezing down to near the water edge. These floating fragments may be water borne to new sites and, on strandage, strike root and become established. In order to lessen dissemination by such freezes, it is desirable to remove either the plants from the water or the water from the plants during the winter period. Alligator weed, water willow weed, and other aquatic plants sometimes become established on mats of drift and flotage. In such situations they are similar to the free floating-mat type of vegetation.

The water schedule on a main river reservoir may involve contours of only a few feet vertically. On a flood control storage reservoir the water level recession zone may be as much as 60 feet or more. In some situations filling the pools early in the growing season and maintaining them in this condition until near midsummer bypasses the establishment of some of the more objectionable emersed species. In the flood control storage type of reservoir with steep shorelines and wide drawdowns the topsoil often sloughs off, exposing the parent material. The dewatered zone of these types of reservoirs are commonly devoid, or essentially so, of littoral vegetation. Littoral plants on these types of pools are generally limited to small areas where tributary streams enter the reservoir.

Coppice of many species of trees, shrubs, and vines can be readily controlled by flooding for varying periods of time, depending on the water tolerances of the species. Intolerant species may be controlled with as little as 30 days of continuous inundation with water 1 foot or more deep. These species usually succumb regardless of whether or not foliar shoots project through the water surface into the atmosphere. Water-tolerant species, on the other hand, may survive a period of flooding of 2 years or more if their leafy stems project above the water surface. Cutting these plants, such as black willow, so the cut portions will be continuously under water even for only 3 months in the growing season gives good control by flooding. Flooding is effective during the growing period but usually has no apparent adverse effects during the dormant winter period. Even small loblolly pines have been observed to survive complete inundation during portions of the winter period.

Flooding has also found use in the control of weeds in agricultural lands (79). For this, small dikes are built around the fields and the area inundated to desired depths and period for control. An important feature is to keep the plants continuously inundated after the area is initially flooded, until the plants are controlled. One or two months of continuous flooding may be required.

Fish ponds and waterfowl food ponds may be operated on an alternate schedule, one pond being used while the other is dewatered, to control aquatic weeds. For providing waterfowl food, crops of millet, buckwheat, and various grains are commonly grown on dewatered sites. These areas are gradually flooded during the winter period or migratory season for use by waterfowl.

2. Shoreline Alteration

In some cases it may be possible to lessen markedly the extent of littoral plants by deepening and filling shallow areas. This procedure may be used in the construction of reservoirs and of fish ponds (49,93). The depth of cutting and filling varies from 1½ to 3 feet. This procedure completely eliminates shallow water areas and may shorten the shoreline to less than one-half of its original length (93). The cut area may remain free of vegetation or sometimes may be colonized by deeper water plants such as buttonbush and water willow weed. Other measures are required for the control of the deeper water plants in these situations.

D. BIOLOGICAL

The expression "biological control of plants" is subject to various interpretations. Here it is used to mean the planned, deliberate introduction or encouragement of an organism in the local environment for selective control of the undesired plants or weeds. The introduced organism may be a pathogen, an insect, fish, turtle, grazing mammal, or even one other plant species.

The potency of pathogens is indicated by the "wasting disease" of eel grass (*Zostera marina*) along the Atlantic Coast of the United States. The "disease" appeared about 1930, and in a few years eel grass disappeared from extensive coastal areas. The notable decrease in the plant was thought to be due to a combination of changes in the environment and to the mycetozoan parasite (*Labyrinthula*), to a fungus (*Ophiobolus*), and to bacteria (67).

Hee (36) has found herbivorous fish effective for the control of aquatic weeds of drains and ditches in Hawaii. This fish, *Tilapia mossambica,* a native of Africa, was introduced deliberately for the purpose of aquatic weed control. A mouth breeder, it scoops mud with its mouth and forms saucer-like depressions in the bottom from 6 inches to 3 feet in diameter, which serve as nests. This habit, coupled with its being herbivorous, has resulted in excellent control of aquatic weeds in drains and ditches of a sugar plantation in Hawaii. Prior to successful use of *T. mossambica* the possible use of live sea turtles for control of aquatic weeds was also explored in Hawaii (36). This species has been successfully used in the United States, Indonesia, and the Philippines for the control of filamentous algae (87). Swingle reported that *Tilapia melanopleura* eliminated filamentous algae and rooted aquatics in the Belgian Congo (87).

The possible use of herbivorous fish has received little attention in the United States since herbivorous species are not native to this country (87). All species reported in the literature are native to Asia or Africa. In the United States, common carp, *Cyprinus carpio,* may control some aquatic plants by keeping the water muddy and to a lesser degree by rooting out plants. In China, Japan, Israel, and Thailand, the grass carp, *Ctenopharyngodon idellus,* has been used successfully for the control of rooted aquatics (87).

In some instances, heavy grazing and trampling by cattle may control plants such as cattail. It appears that marginal grazing should be encouraged where it fits into the over-all land-use program (93). The introduction of nutria, or coypu, in the southeastern United States might present a threat to valuable muskrat and waterfowl feeding grounds in the coastal marshes or might become a pelt contributor of real economic value (1,96). This mammal has been described as a "four-legged weedcutter" and a warning issued that it could become a serious economic pest (1). Nutria often devour palatable farm crops and become another nuisance to the farmer (1,96).

The blanketing effect of algae to exclude light from the lower levels is a principle applied for the practical control of submersed weeds in fish ponds by Swingle and Smith (88). This may be accomplished by the proper application of commercial fertilizer which promotes the growth of fish foods and provides plant control. The fertilizer may be applied from the pond bank, from a boat, or by means of special platforms (50,88). This method of control may be used to prevent the establishment of underwater weeds in new ponds or to control such weeds in old infested ponds (88). In this method, lower plants, or algae, are utilized for the control of higher plants. Draining, raking, and other mechanical means were costly and relatively ineffective on underwater weeds in ponds (88).

The use of plantations of water-tolerant woody species also offers real possibilities in some situations for the control of aquatic plants (82). By providing a shade canopy and competing for space, plantations of bald cypress and tupelo gum may practically exclude such aquatics as water willow weed, cattail, and other emersed species. Marginal bands of dense stands of black willow and buttonbush sometimes practically exclude sedges, grasses, and forbs from the understory.

Oborn has pointed out the possibility of using *Eleocharis acicularis* and *Sagittaria subulata* for crowding out taller pondweeds in irrigation ditches (71).

From the foregoing, it appears clear that biological control of aquatic weeds offers real possibilities. In general, satisfactory biological control methods of wide application still remain to be developed. Because risks are involved in the introduction of a species for plant control, or any other purpose, such attempts should be made only if careful study of each specific case indicates the risk is merited. The fundamentals of biological control and examples of terrestrial plant species effectively controlled in this manner have been summarized recently (43).

E. LEGAL

Legal control of aquatic plants involves stringent quarantine laws to prevent the introduction and distribution of noxious species to uninfested areas.

IX. Control versus Eradication

Certain species of aquatic plants can be effectively controlled; for other species practical control measures are lacking. The control of a plant implies a reduction in the area it covers to the extent that it does not offer a serious problem. The species may still be present in the area and may sometime in the near future recolonize former areas and again require control. In waterways, where water-borne reproductive structures occur, colonization is a continuing process. Water-borne reproductive structures move mainly downstream. Control procedures should proceed from upstream to downstream to lessen new infestations downstream. Some aquatic plants, such as lotus and water hyacinth, can be controlled readily and economically with herbicides.

The eradication of a species from a natural geographical area, such as a large watershed or drainage basin, may or may not be economically practical; in many cases it may not be possible. Eradication means the elimination of the species from the natural region or drainage basin. Before attempts are made to eradicate a species from a natural geographical area, a highly effective method for control of the plant would be a prerequisite. The growth habits of the plant should be well known, including such items as methods of reproduction, migration, and establishment. The extent of colonization and potential areas for colonization also should merit consideration. The most appropriate time to attempt eradication of an introduced species appears to be shortly after its initial introduction. Even more important would be the prevention of its initial introduction by plant quarantine laws.

Regardless of the source of the species, whether introduced or native, certain features of its growth pattern and life history may influence the possibilities of eradication. For example, the water chestnut (*T. natans*) is an annual, and most of its seeds germinate in the first spring after they are produced (60). Although some of its seeds are reported to remain viable under favorable conditions for 5 years or more (59), the seeds lose their viability out of water (67.) This feature reduces the possibility of introducing water chestnut into new areas by being transported by man since the air-dried caltrops are sold at fairs and elsewhere as curiosities. This plant occurs mostly in open water areas where it is relatively easy to locate. If an effective herbicide could be applied during the flowering period to inhibit seed production over several successive seasons, this plant might be eradicated from important watersheds. Lotus colonies can be readily controlled, but the fruits are extremely long-lived. Museum records show viable periods of more than 50 years for *N. lutea* and more than 150 years for *Nelumbium* (8), and circumstantial evidence indicates a seed longevity for *N. nucifera* of at least 200 years in Manchuria (72). Because of the great longevity of lotus seeds, eradication of this species from even a locale does not appear feasible. Alligator weed reproduces by floating nodal fragments. Seed production of this species in the southern United States is not known to occur. The vegetative reproductive fragments float, and with the recession of floodwaters they may be stranded and strike root over extensive vegetated areas. Because of this mode of dissemination and the establishment of colonies in vegetated areas, it is impractical and, perhaps, not possible to find all the plants for treatment. There is also a need for a highly effective herbicide for control of alligator weed and water chestnut. Water hyacinth may be effectively controlled with 2,4-D. The eradication of water hyacinth from the United States appears desirable, but its control over more extensive areas must precede such a remote possibility.

For most aquatic species control will be more realistic and economical than attempts at eradication. It may be feasible to eradicate an introduced species from a large drainage basin or watershed especially if the plant is found in the early stages of colonization. For most species however, eradication from the natural geographical region will result only in control. Control efforts are much less costly than eradication attempts. The possibility of reintroduction of the species should also be considered before undertaking eradication.

X. Suggested Guidelines for Plant Control

Some suggested guidelines as to the types of information needed for successful control of a plant may be helpful. Information on the growth form, ecological anatomy, physiology, and water level relationships of the plant should be helpful in guiding control techniques. In order to control a given species, reliable answers are needed to the questions of "what, when, where, and how?" concerning possible control measures for that species. What measures can one use effectively and economically—mechanical, herbicidal, manipulation of water levels, biological? When should the method of choice be applied—frostless season, freezing period, or in a certain phenological aspect? Where with respect to the plant organs should the control measure be directed—roots, stem, leaves, flowers, fruits, seeds? How should the operation be carried out—by hand, by boat, by aircraft, by gates, by bottom drains, or in design? Reliable answers to questions of this sort, secured under local conditions, should be helpful in ascertaining whether or not a practical method of control is available in that particular region. The effectiveness of a control measure may vary with local conditions. Environmental factors—chemical, physical, and biotic—influence the effectiveness of control measures. The more important of these appear to be water aeration, temperature, and phenological aspect. Answers to the question "why" should be sought in order further to refine control procedures.

Once a practical control procedure has been determined, there are four steps in placing a species under control and maintaining it in this condition: (1) properly define the locations and extent of the plants to be controlled, (2) apply an effective control method, (3) maintain surveillance in the area under control for locating resprouting colonies or further colonization, and (4) make early retreatment of the new growth. Following these suggested guidelines should lead to effective field measures for the control of aquatic plants.

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A DIGEST OF AVAILABLE INFORMATION ON THE INSECTICIDE MALATHION

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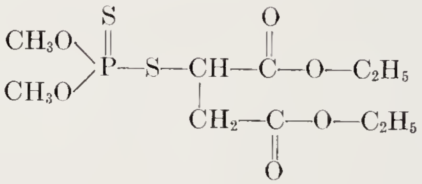
I. Properties of the Insecticide

A. INTRODUCTION

Malathion is the coined name approved by the U. S. Interdepartmental Committee on Pest Control and the Committee on Insecticide Terminology of the Entomological Society of America for a chemical which is the *O,O*-dimethyl dithiophosphate of dimethyl mercaptosuccinate, variously referred to as compound 4049; malathon; *2(1,2 dicarbethoxyethyl) O,O-dimethyl phosphate; 2-1:2 di(ethoxycarbonyl) ethyl dimethyl phosphorothiolothionate; O,O-dimethyl S-(1,2-bis-carbethoxyethyl) phosphorodithioate*, and currently defined as *S-[1,2-bis-(ethoxycarbonyl)-ethyl] O,O-dimethyl phosphorodithioate** (10,546). The accepted structural formula is I.



$$\begin{array}{l} \mathrm{CH_3O} \\ \mathrm{CH_3O} \end{array}\!\!>\overset{\mathrm{S}}{\overset{\|}{\mathrm{P}}}-\mathrm{S}-\underset{\underset{\displaystyle \mathrm{CH_2}-\underset{\underset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{O}-\mathrm{C_2H_5}}{|}}{\mathrm{CH}}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-\mathrm{O}-\mathrm{C_2H_5}$$

(I)

Malathion is a high-boiling liquid, deep brown to yellow in color, slightly soluble (145 p.p.m.) in water, soluble in many organic solvents (e.g., alcohols, esters, ethers, ketones, aromatic hydrocarbons, and vegetable oils), but very slightly soluble in paraffinic solvents (240). Malathion is relatively nonvolatile. The vapor pressure is reported to be 4×10^{-5} mm. Hg at 30°C., a saturated atmosphere containing about 0.5 p.p.m. (227,462). Pure malathion has no unpleasant smell. However, the commercial product, which contains 95% active ingredient, has a markedly unpleasant odor, but this usually disappears within 24 hours of application. A premium grade product with a less marked odor is available.

Malathion was announced as an insecticide in December, 1950, by the American Cyanamid Company who claimed it to be less toxic to mice and rats than parathion, although approaching parathion in insecticidal activity. These claims have been largely substantiated, and malathion now enjoys wide use as an insecticide.

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* A succinct statement regarding naming of organophosphorus compounds appears in *Rev. Appl. Entomol., A43,* 69 (1955). Perusal will make it clear why malathion is called a "dithioate" rather than a "thiolothionate." Sometimes the phosphate moiety is termed "dithiophosphate" (e.g., 188).

Two analytical methods have been proposed: a polarographic method for process control and for determination in technical products (312) and a micro method suitable for analysis of technical products and malathion residues on and in plant and animal products. In the micro method malathion is removed by extraction with carbon tetrachloride, decomposed by alkali, extracted, and converted to an intensely yellow copper salt soluble in carbon tetrachloride which is measured at 418 μ. Amounts between 0.25 and 2.5 mg. in 100 ml. of extract can be readily determined. Recoveries range from 60 to 100% for surface residues on fruit and vegetables and from 60 to 90% for residues in chemical products (218,446).

The micro method has been tested collaboratively, studied, elaborated, and modified by the Association of Official Agricultural Chemists, who now provisionally accept it for evaluation of surface residues on fruit and vegetables. Recoveries of surface deposits were within 5% of added malathion. However, when malathion was distributed throughout the sample, recoveries were as low as 55%, but were much improved if the initial extraction was made with alcohol and this extract was then diluted and re-extracted with carbon tetrachloride for analysis (103).

The original method (446) has been adapted to analysis of malathion in animal products, amounts as low as 20 μg. of malathion being determined by the revised procedure. Recoveries range from 67% in beef to 132% in raw whole milk, but in general the method appeared satisfactory for determining malathion in fat, meat, milk, eggs, and liver in concentrations down to 1.0, 0.5, 0.02, 0.5, and 1.0 p.p.m., respectively (445). With fresh whole milk the sensitivity is 0.01 p.p.m. (231).

Organophosphorus insecticides, including malathion, can be satisfactorily run and detected on paper chromatograms. For chromatography mineral paraffin is used as a stationary solvent and ethanol–acetone–water (1:1:2) as the mobile phase. Malathion runs with R_f 0.93, while EPN, parathion, Diazinon,* Chlorthion,** and methyl parathion are slower and well separated (105). Spots may be detected by brominating the chromatogram with bromine vapor or *N*-bromosuccinimide and detecting the excess bromine with fluorescein. The spot is then located by the highly fluorescent yellow-green of fluorescein on the pink background of brominated fluorescein. A positive test can be obtained with 2 μg. of malathion (104). If water is used as the mobile phase, most organophosphorus insecticides, including malathion and Diazinon, remain at the origin, but the inhibitors present move along the paper and may be detected by overlaying the chromatogram with a paper containing cholinesterase (human blood plasma) and bromothymol blue and a second paper containing acetylcholine. Acid is liberated during the enzymic breakdown of acetylcholine, and thus inhibitors are indicated by unchanged blue areas on a yellow background. The method is of great sensitivity, detecting the inhibitor in as little as 0.02 μg. of Diazinon (106).

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* Registered trademark Geigy Chemical Corp., Ardsley, N. Y.

** Registered trademark Chemagro Corp., Kansas City, Mo.

B. VERTEBRATE TOXICITY

When malathion poisoning is induced in rats, mice, guinea pigs, cats, dogs, sheep, or calves, the symptoms are typical of those produced by other organophosphorus insecticides and are characteristic of the anticholinesterase activity (43,121,270,481). The signs are of short duration, and unless death occurs within a few hours recovery appears to be complete (270). Sheep and calves generally first show excessive salivation. The animal usually then encounters respiratory difficulty, breathing with the mouth open and with grossly exaggerated respiratory movements. As the respiratory effort increases, the animal walks stiff-legged and wanders aimlessly. Eventually exhaustion forces the animal to lie down. As death approaches, there are loud rales from the lungs and the animal grunts softly. Death appears to be due to suffocation. Only with the highest doses have convulsions been seen (481). With dogs (43) and cats (121) the picture is entirely similar. Lethal doses produce periods of restlessness and mild depression, disorientation and unsteadiness, involuntary urination and defecation, vomiting, and deepened respiration, followed by rapid shallow respiration, muscular fibrillation, prostration, and death. The signs of malathion toxicity shown by chickens include drowsiness, incoordination, excessive salivation, diarrhea, coma, and death; there are no signs of increased excitability or uneasiness (199,536). Fish become lethargic and do not swim when touched; in severe poisoning the body may be bent laterally (125).

Frequently autopsy findings are entirely negative, and even in cases of acute poisoning the lesions found are never outstanding and never pathognomonic (121,198,199,270,481,536). For example, a diet containing 10,000 p.p.m. of malathion caused death of all fowls, but none showed nerve damage on histopathological examination (190).

Perfusion of the isolated rabbit heart with $3 \times 10^{-5}M$ malathion decreased heart rate, coronary flow, and amplitude of contraction; atropine did not afford protection against these effects (43). When applied to isolated rabbit or guinea pig ileum, $1.2 \times 10^{-4}M$ malathion induced decreased tone until complete relaxation and cessation of mobility amounting to flaccid paralysis were attained. This paralysis appeared to be unrelated to cholinergic effects, for it did not respond to atropine, nicotine, acetylcholine, or barium chloride and could not be prevented by prior administration of atropine, nicotine, or the anticholinesterases neostigmine or diisopropyl fluorophosphate. The evidence suggests a depressant action on muscle metabolism rather than a neurohormonal mechanism (43).

With normal animals no toxic symptoms were produced by single oral doses of 10 mg./kg. for baby calves; 50 mg./kg. for 1–2-year-old sheep (481); 90 mg./kg. for white rabbits (3); and 100 mg./kg. for 3–4-week-old chickens, although some yearling birds were killed by the same dose (199). Some calves were severely affected by but survived 20 mg./kg., one sheep was killed at 150 mg./kg. (481), and a white rabbit failed to survive 1000 mg./kg. (3). Dogs survive very large doses: 2000 mg./kg. induced mild cholinergic symptoms, 4000 mg./kg. was emetic (188), and 3500 mg./kg. was without effect provided vomiting was prevented by total anaesthesia (53). A cat survived a single oral dose of 500 mg. (121).

The acute oral LD_{50}'s have been determined for a number of animals and are given in Table I. Some similar data for parathion are included for comparison.

The chronic oral toxicity is so slight that it has yet to be determined for any vertebrate. White rats maintained on diets containing 100, 1000, and 5000 p.p.m. of malathion showed steady weight gains and food consumption and survived as well as or better than the controls over a 2-year period (240,250,270). Hens on a regimen containing 50 (602) or 100 (394) p.p.m. of malathion showed no ill effects: 10,000 p.p.m. was lethal to all birds (190).

Acute dermal toxicity is very low. For single applications it lies between 2460 and 6150 mg./kg. for white rabbits (240) and at

TABLE I

Acute Oral LD$_{50}$'s of Malathion and Parathion to Some Vertebrates

Animal	Parathion LD$_{50}$, mg./kg.	Malathion			
		Formulation	Vehicle	LD$_{50}$, mg./kg.	Ref.
Rats	11	—	—	1500	165
Rats, male	—	98% Tech.	Corn oil	1400 ± 100	188
		90% Tech.	Corn oil	480	270
		99+%	Corn oil	1845	270
		65% Tech.	Propylene glycol	369	270
		90% Tech.	Propylene glycol	1156	270
		—	Undiluted	5843	20
	13	—	—	1375	22
	—	—	—	1500	238
Rats, female	3.6	—	—	1000	22
	—	—	—	1200	238
		65% Tech.	Propylene glycol	739	270
Mice	—	—	—	1120	238
	9.7	—	Corn oil	930	308
	12	—	Corn oil	775	270
Mice, male	—	90% Tech.	Corn oil	886	270
		99+%	Corn oil	3321	270
		65% Tech.	Propylene glycol	1260	270
		25%^{a}	—	1025	270
		—	Undiluted	4059	270
Male, female	—	65% Tech.	Propylene glycol	1158	270
Guinea pigs	—	—	—	570	238
Chickens					
2–3 weeks	—	—	—	370	536
3–4 weeks	—	—	—	200–400	199
1 year	—	—	—	150–200	199
Ducks	—	—	—	1107	158

^{a} Wettable powder %.

4444 mg./kg. for male and female white rats (20). For chronic applications, four or more daily doses of 1230 mg./kg. were required to produce mortality (270). Dermal applications of single sprays of 0.25 and 0.5% wettable powder did not affect baby calves, but at 1% 3/4 calves died. When the malathion was applied as an emulsion at this concentration, 21/21 calves were unaffected (481). Also unaffected were cattle sprayed at 1.25% (356), dogs immersed in 5% dips (474) or dusted with 4% dusts (247) or dipped four times at

4 day intervals in a 2% emulsion (53), cats powdered daily for 14 days with 25% dust or dipped in 0.22% emulsion (121), and fowls sprayed individually with 38 ml. of 0.5% malathion (394) or dusted weekly for 4 weeks with a 4% malathion powder (199). Also unaffected was hatchability of eggs from hens kept in pens where litter had been dusted with 12.5 lb. of 4% malathion dust per 1000 square feet (258). Fowls showed abnormality but recovered when dipped in 1% malathion emulsion, although turkey poults were unaffected; at higher concentrations some or all were killed (198).

The toxicity of insecticides by injection is of less interest than the oral or dermal toxicity, but has sometimes (e.g., DDT) served to indicate barriers to penetration from either skin or gut. For guinea pigs the acute intraperitoneal LD_{50} of 500 mg./kg. contrasts greatly with the 12 mg./kg. for parathion. For mice the acute intraperitoneal LD_{50} is 815 mg./kg. (451). Hens injected intraperitoneally with malathion showed signs of distress, but even at 200 mg./kg. they were not prostrated or killed and at 17.5 hours after injection appeared normal (394). When malathion was injected subcutaneously into atropinized hens the LD_{50} was 1400 mg./kg., but a number of hens given high doses continued to lose weight for up to a month after the acute signs had passed (167). To male white rats the acute subcutaneous LD_{50} was 1000 mg./kg., compared with 6 mg./kg. for parathion (344). However, from intravenous application the LD_{50} to white rats was about 50 mg./kg. (238), suggesting that the potency of malathion given intraperitoneally or subcutaneously is reduced by barriers not met on the intravenous route.

There is one undoubted case of malathion poisoning in man, but the circumstances are so unlikely that it is worth quoting them in full (611).

> On June 27, 1956, at 5:30 P.M., a housewife, aged 35 years, discovered that she was suffering from toothache after consuming a small amount of alcohol. In order to alleviate the pain she resorted to the unusual expedient of swabbing the offending tooth with malathion fly spray. Because of the lack of analgesic response she drank the remainder of the malathion, approximately 16 oz. in quantity. Fortunately she had told her husband of her actions and three hours later she was brought to the casualty department of the Royal Perth Hospital.

The fly spray was stated to comprise 1.5% technical malathion plus an equal amount of toluene-xylene solvent and a nonionic emulsifier. The dose ingested would therefore be approximately 6.75

g. of malathion, which might (the weight of the woman is unknown) be about 130 mg./kg. The woman lost consciousness, defecated and micturated involuntarily, and regurgitated a great quantity of dark fluid containing particles of food. Atropine was administered, and within 4 hours of the ingestion of malathion the patient had recovered consciousness. The following morning there were no significant findings attributable to the toxic episode. The relevant features of the illness were comparable with manifestations of mild to moderate intoxication due to other organic phosphates such as parathion and HETP (611).

An illness of a 10-year-old girl has also been attributed to malathion poisoning (460). However, the evidence for involving malathion is quite unconvincing, and it has been concluded that an infection was the etiological agent (167).

It is unlikely that health hazards will attend the normal usage of malathion. A study made during a community-wide airplane spraying at 0.5 lb. of malathion per acre showed that the total inspiratory and skin exposure of a man walking outdoors was 1/500,000–1/125,000 of his probable LD_{50}. Thus there is no hazard to the normal human population (83). Operators applying and hence exposed to malathion aerosols in the field for two weeks were found to inspire some 11–21 mg. of malathion, a further 32–86 mg. being deposited on the skin. From these data it was computed that the probable monthly dose was 100–200 times less than the acute LD_{50}, indicating that community-wide application of malathion aerosols presents no hazards even to operators of aerosol generators (122).

However, the use of malathion with other organophosphorus insecticides, especially EPN, may be hazardous. When malathion is administered with Systox,* parathion or methyl parathion, their effects are additive. When malathion is administered with EPN, there is considerable potentiation or greater than additive effect (635). For single doses the potentiation was about 50-fold, as little as 100 mg./kg. of malathion plus 2 mg./kg. of EPN killing 4/4 dogs (188). And the LD_{50} was halved when malathion was administered to white rats which had been injected 1 hour previously with 1.5 mg./kg. of EPN (436). Dogs fed for 6 (636) or 12 (188) weeks on a diet containing legal tolerance levels for malathion (8 p.p.m.) and EPN (3 p.p.m.)

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* Registered trademark Chemagro Corp., Kansas City, Mo.

had normal plasma and red blood cell cholinesterase; when the diet contained 100 p.p.m. of malathion and 20 p.p.m. of EPN—the so-called safe levels or highest no-effect concentrations—plasma ChE was not affected, but red blood cells ChE decreased precipitously, indicating definite potentiation. When the diet contained 250 p.p.m. of malathion and 50 p.p.m. of EPN, plasma ChE was inhibited up to 60% and erythrocyte ChE up to 93%, but the animals did not exhibit gross symptoms of poisoning (188).

Although the signs and symptoms of malathion poisoning are characteristic of anticholinesterase activity, it is now clear that pure malathion is not itself an anticholinesterase; rather, it is converted to an active anticholinesterase by the animal (417). As supplied, malathion may cause inhibition of ChE *in vitro*, but the inhibition is mild and varies considerably from sample to sample. It is therefore likely that the inhibition is due to impurities (179). The actual identity of this inhibitor has not been established. It may be the isomer of unknown constitution caused by prolonged heating of malathion. This material (or materials) is a substantially stronger ChE inhibitor than malathion (416,448), and it has been suggested that the *in vitro* inhibition of ChE obtained with some samples of malathion is to be attributed to isomerization on aging (263,416).

Dilute bromine water instantly converts malathion solutions to a potent ChE inhibitor, different malathion samples having the same potency after conversion; *N*-bromosuccinimide has also been employed (107), but excess of this reagent interferes with the enzymic determination of inhibitor, while excess bromine does not (179). Aerobic incubation with tissue may markedly increase the anti-ChE effect (108,450). Very likely the active inhibitor produced in these preparations is malaoxon (448,450), the oxygen analogue of malathion, which is several hundred to many thousand times more active than malathion against mammalian and insect cholinesterase (394,450).

When malathion is administered, there is rapid production of inhibitor. In rats sacrificed 2–5 hours after oral administration of an LD_{75} dose, brain ChE was 35% inhibited, erythrocyte ChE 47% inhibited; with smaller doses inhibition was less (238). This ChE inhibition may endure far beyond any gross evidence of toxicity. In rats, where ChE inhibition had been induced by a massive intraperitoneal dose of malathion, plasma and brain had returned to normal after 12 days, red cells after 28 days. With guinea pigs, plasma ChE had returned to normal in 10–19 days, but red cell and brain ChE still showed some inhibition after the 19 day interval (270).

Likewise there can be considerable inhibition of ChE without precipitating signs of poisoning. This can occur with single doses, but is most marked during chronic exposure or ingestion. Spraying cows with 4.5 or 9 g. of malathion daily, or 9 g. every third day, markedly and progressively reduced the erythrocyte ChE without affecting either milk yield or body weight. Regeneration of ChE activity started shortly after the final application of malathion (621). When rats were fed 5000 p.p.m. of malathion in the diet for two years, the ChE inhibition in plasma was 80%, in red cells 100%, and in brain 77%, yet there were fewer deaths than in the controls (270). The importance of such covert inhibition is that it is possible to lower the ChE reserve progressively to a threshold beyond which poisoning will be induced by minute additional amounts of inhibitor. Thus animals maintained alongside of or on frequently treated fields or pastures may gradually have their ChE reserve reduced to the vanishing point, thereby increasing their susceptibility to cholinesterase inhibitors as well as to cholinergic drugs which may be employed in the therapy of certain conditions (481). Obviously, depletion of ChE reserve by chronic intake is less likely to occur with malathion than with more potent inhibitors, but the possibility cannot be overlooked, for livestock do not avoid malathion-treated foliage; indeed, there is a record of a malathion-treated area being preferentially grazed by cattle (543).

The low toxicity of malathion to vertebrates is not due to failure of the insecticide to penetrate skin or gut, although in ruminants significant amounts may be detoxified by rumen fluid before absorption can occur (5). Malathion sprayed on calves was rapidly absorbed and eliminated, principally as water-soluble metabolites and degradation products in the urine. The amount eliminated was greatest during the first 24 hours after spraying and then decreased; when the animal was sacrificed at 2 weeks after spraying, only 2.7% of the applied dose was recovered from the whole hide (395). When hens were fed a diet containing malathion, 60% of consumed insecticide was eliminated in the droppings in 2–4 days, almost all as water-solubles, indicating that the malathion had been degraded before elimination. Intraperitoneally injected malathion suffered the same fate; more than half was eliminated in 3 hours and nearly all in 24 hours, again mainly as water-soluble degradation products (394). When hens were sprayed, less than 12% of the applied dose was eliminated in 32 days, suggesting that malathion is not absorbed from feathers (394).

In the whole animal, breakdown of malathion is rather complex, chicken droppings and mouse urine containing at least seven distinct compounds after intraperitoneal injection of malathion. It was an early suggestion that breakdown follows two routes: one route probably involves oxidation of the thiono-sulfur moiety to produce a series of thiolphosphates, one of which, malaoxon, may be the metabolite which is principally responsible for anticholinesterase activity and hence toxicity; the second route probably involves progressive hydrolysis of the ethyl ester moieties to produce water-soluble compounds having low cholinesterase activity and which are relatively nontoxic (394). As malaoxon is also rapidly broken down via the second route (436), the two pathways may not be independent.

In the American cockroach, malathion metabolism is apparently much simpler and less extensive than in warm-blooded animals (394). The existence of alternate or additional metabolic pathways bypassing or detoxifying the highly toxic thiolphosphates probably accounts for much of the great differential toxicity for malathion between insects and warm-blooded animals. However, mice given massive oral doses of malathion are rendered transiently toxic to the mosquito *Aedes aegyptii* (274), implying that malathion or inhibitor occurs in the blood and suggesting that part of the reason for the low mammalian toxicity must reside in a slower or localized conversion to anticholinesterase or in the relative ineffectiveness of inhibitor for mammalian ChE.

Some of the activation and much of the degradation probably occur in the liver. In mouse liver preparations, the initial rapid activation of added malathion was opposed by a vigorous degradation, and after 15 minutes the anticholinesterase activity decreased steadily (450). Rat liver homogenates hydrolyzed malathion very rapidly to a noninhibitor, the monoacid derivative of malathion, formed by removal of an alcohol group from the diethyl succinate portion of the malathion molecule (108–110). The enzyme responsible, called malathionase by Cook and Yip (110), occurred in the six tissues examined: liver, kidney, serum, lung, ileum, and spleen of rats, guinea pigs, mice, and dogs except that activity was very low in the serum, ileum, and spleen of dogs and in the spleen of mice. In each species maximum activity was in the liver, the liver of dogs having a particularly high activity (436). This enzyme system also hydrolyzes other esters; greatest affinity is for triesters, although diesters and to less extent monoesters are attacked; acetylcholine is unaffected (651).

Malathionase does not attack other organophosphorus insecticides (except malaoxon (436)), but is very rapidly inhibited by small amounts of their oxidized compounds, all being about equally effective malathionase inhibitors, in striking contrast to their wide range of potency as ChE inhibitors. This difference in inhibitory activity on malathionase and cholinesterase has therefore been invoked to explain why parathion, for example, is not an *in vivo* "potentiator" of malathion toxicity, even though it is a strong inhibitor of malathionase. Parathion is so much more potent as an inhibitor of cholinesterase than as an inhibitor of malathionase, that, *in vivo*, its toxicity due to ChE inhibition is fully manifest before sufficient malathionase is inhibited to interfere with the rapid detoxification of malathion. On the other hand, EPN, considerably less effective than parathion as an anticholinesterase, is about equally effective against malathionase; hence a dose insufficient to inhibit ChE may inhibit malathionase and prevent detoxification of malathion (109). When rats were injected with half the LD_{50} of EPN and sacrificed 1 hour later, malathionase of serum and liver was completely inhibited. With smaller amounts of EPN, inhibition of malathionase was less complete. Slow recovery of malathionase activity occurred during the following days (436). When intake was continuous in diet for 2 weeks, the amount of inhibition was dependent upon the EPN content of the diet, the highest level (100 p.p.m.) causing almost complete inhibition. The lowest level of EPN capable of producing significant ChE inhibition caused more than 80% inhibition of malathionase (189,436).

A few organophosphorus compounds may cause a delayed paralytic effect, the so-called "ginger paralysis" or "Jake leg," which is essentially irreversible. Hens dosed with malathion sometimes develop leg weakness in addition to more usual symptoms. The muscle weakness was localized, persisted after acute symptoms had vanished, and, while present, was clinically indistinguishable from "ginger paralysis." However, it differed from "ginger paralysis" in that the sign disappeared completely within 4–21 days. The minimum dose producing muscle weakness was 100 mg./kg. (167). A yearling hen survived a single oral dose of 200 mg./kg., but was still lame after 3 weeks (199).

To safeguard the consumer, legal tolerances for malathion residues have been established. Additionally, recommendations have been made which, when accepted, safeguard the health of user or consumer.

In the United States zero tolerances have been established for milk and eggs; 2 p.p.m. for corn and cotton seed; 4 p.p.m. in or on meat and meat by-products; 8 p.p.m. for a very wide range of fruit and vegetables and from post-harvest application to grains in storage (190a). A tentative threshold for malathion in air is 15 mg./m.^{3} (22).

In Canada 8 p.p.m. has been established for a wide range of fruit and vegetables (27) and for grain in storage (17).

Recommendations in Great Britain concerning use of malathion for control of stored products pests state that there should be no hazard to consumers provided (*a*) the amount admixed with small grains or oil seeds does not exceed 10 oz. per bushel (approximately 10 p.p.m. for wheat), (*b*) the amount added to the surface of bulk grain or oil seed does not exceed 5 oz. per 1000 square feet of surface, and (*c*) the amount deposited on the surface of stacks of grain or oil seeds in closely woven bags does not exceed 114 mg. per square foot of surface. Malathion is also permitted for treatment of walls and surfaces of empty storage rooms, wagons, and storage bins and for the surface of stacks of foodstuffs in woven or fiber containers (25).

All these tolerances and recommendations are probably ultraconservative in view of the virtual nontoxicity of malathion to vertebrates, 100 p.p.m. on the whole diet being accepted as the highest no-effect, or safe, level (636).

C. INSECT TOXICITY

Laboratory and field trials have established that to a number of insects and mites malathion has about a quarter the insecticidal efficacy of parathion, but there are wide variations from species to species, and these are sometimes equalled by differences found in

TABLE II. Arthropod Toxicity of Malathion Relative to Parathion (Based mainly on LD$_{50}$)

Species	Stage	Relative toxicity (parathion = 100)	Ref.
Tetranychus bimaculatus		27; 41	142
	Adults	224[a]; 107[b]	170
	Larvae	178[a]; 87[b]	170
	Eggs	59[a]; 44[b]	170
Locusta migratoria	Adults	3	379
		5.1	573,574
Locusta gregaria	Adults	3	379
Blattella germanica	Adults	7	308
Aphis rumicis	—	25	308
Therioaphis maculata	—	63[c]	48
Chaitophorus populi	—	36	142
Myzus persicae	—	~25	68
		41; 21	142
Oncopeltus fasciatus	—	74	308
Orius insidiosus	Adults	45	79
Listroderes obliquus	Adults	4	52
Sitona cylindricollis	Adults	53	9
Sitophilus granarius	Adults	48; 45	142
		~15	601
Tenebrio molitor	Larvae	<10	142
Tribolium confusum	Adults	24	308
	Adults	7; 9	142
	Adults	25	601
	Larvae	5	601
Trogoderma granarium	Larvae	75[c]	367
Ephestia kuhniella	Larvae	<5	142
Porosagrotis orthogonia	Eggs	~25	180
Protoparce sexta	Larvae (large)	11; 4[d]	235
	(medium)	16	235
	(small)	12	235
Lucilia cuprina	Larvae	<1	168,184
Musca domestica	Adults	4.3	142
		4.6	54
		6.2	217
		4.2	185
	Larvae	14	563
Ceratitis capitata	Adults	55[e]	600
	Larvae	25	535
Dacus cucurbitae	Larvae	11	535
Apis mellifera	Adults	6; 8; 11[d]	54
		8; 21; 38	

[a] Emulsions. [b] Wettable powders. [c] LD$_{95}$. [d] As stomach poisons. [e] Oral, sure lethal.

a single species by the same or different investigators. Available information is assembled in Table II.

Table II shows that although malathion was more toxic to adults and larvae of *Tetranychus bimaculatus* than was parathion, there is no reported case where tests have established that an insect was more susceptible to malathion than to parathion. It is therefore a most interesting observation that one species of insect not controlled by parathion is controlled by malathion. In some citrus orchards in California outbreaks of soft scale, *Coccus hesperidum,* have followed applications of parathion for control of other pests. These outbreaks apparently result from the adverse effect of parathion on entomophagus insects, principally *Metaphycus leuteolus,* which normally keep populations of soft scale at a low level. Malathion, either alone or applied with parathion, effectively prevented these outbreaks (174).

Some of the information on toxicity of malathion relative to insecticides other than parathion comes from studies on strains of house fly more or less resistant to other insecticides (82); obviously, the relative toxicity figures are immediately biased by this resistance. Additionally, with strains of house flies not known to be resistant to malathion, the LD_{50}'s range from 0.16 to 1.07 μg. per fly (82,453,617), with some evidence that strains resistant to other insecticides may show cross tolerance to malathion (482). These factors aside, DDT is about 16 times as toxic as malathion to nonresistant house flies. The deer fly, *Chrysops discalis,* is 6 times more susceptible to DDT than to malathion (518). In contrast, female *Anopheles quadrimaculatus* are 2.3 times, males 7 times, as susceptible to malathion as to DDT, the sex divergence resulting from the greater susceptibility to DDT, not to sex differences in susceptibility to malathion (377). With the lesser house fly, *Fannia cannicularis,* the male is more susceptible to malathion than the female, but both are more than 20 times as susceptible to malathion as to DDT (357). Nymphs of the scale, *Drosicha mangiferae,* are 12 times (473), worker bees 18 times (54), sixth instar larvae of *Heliothis zea* and *H. virescens* 23 and 40 times, respectively, as susceptible to malathion as to DDT (213). Obviously, variation from species to species is such that it is not possible to generalize regarding the relative toxicity of malathion and DDT. Similar considerations apply to other chlorinated hydrocarbon insecticides.

There is little to suggest that malathion is either markedly attractive or repellent to insects. When offered in 2% sugar at the incredibly high doses of 15 and 1.5 g./square foot, malathion was only slightly less attractive than the sugar solution alone. At the more moderate dose of 150 mg./square foot, malathion plus sugar was markedly more attractive than sugar alone (298). But the potato flea beetle, *Epitrix cucumeris*, against which malathion is ineffective, detects and avoids malathion deposits from spray concentrations of about 1–800 (343).

Systemic properties are neither pronounced nor absent. Aphids on the lower surface of leaves may be killed when only the upper surface is sprayed, total kill being obtained at twice the malathion concentration effective for direct spraying (142). With mites the effect is less pronounced, the LD_{50} of mites on the unsprayed surface being some 30 times that of those on the sprayed surface (170). But insufficient malathion is transported in the vascular system for unsprayed leaves to become toxic (382), although it is claimed that when malathion is applied to flowering borage, the nectar is toxic to bees for several days (407).

Sprays may be prepared from emulsions or wettable powders; dusts may be formulated with different carriers. As with other insecticides, each variation may affect toxicity. When malathion was added to the larval food of house flies, emulsions were 1.75 times as effective as wettable powders (563). Malathion sprays prepared from wettable powder gave better control of European corn borer, *Pyrausta nubialis*, than sprays prepared from emulsifiable concentrates. Granules were not more effective than emulsions (114). Emulsions were more effective than wettable powders against the Taxus mealy bug, *Pseudococcus cuspidatae* (620), the purple scale, *Lepidosaphes beckii* (559), and the Indian grasshopper, *Chrotogonus trachypterus* (477), yet wettable powders were vastly superior to emulsions when tested against the Indian meal moth, *Plodia interpunctella* (313).

When malathion dusts formulated with various carriers were tested under standard conditions against a two-spotted mite, *Tetranychus cinnabarinus*, those formulated with calcium carbonate, kaolin, pyrophyllite, talc, and a specially fine grade of attapulgite consistently gave high kills, with diatomite little if at all inferior; dusts formulated with montmorillonite and two dusts formulated on different samples of attapulgite gave much lower kills (552). Perhaps this explains a discrepancy between the laboratory effectiveness of malathion as sprays and as dusts against adult sweet potato weevil, *Cylas formicarius elegantulus*. When applied as a spray malathion was equitoxic with endrin, methyl parathion, and EPN, but when these materials were formulated as dusts on attapulgite clay, malathion was much inferior (428). Against stored products pests, talc was a less effective carrier than kaolin, a difference thought to be due to poor adhesion of talc to grain and insect (462).

The toxicity of malathion increases with temperature (477). In two strains of house flies, toxicity increased about a third for a 10°C. rise in temperature (482); to the boll weevil, *Anthonomus grandis*, doubling the temperature (from 16 to 32°C.) about doubled the toxicity (655).

Among organophosphorus insecticides, malathion is unusual in that death occurs late following a greatly prolonged ataxic phase. In cockroaches onset of ataxia is moderately rapid, occurring within 30 minutes to 1 hour, yet is extraordinarily prolonged so that at doses of 10 μg./g. some of the cockroaches were completely moribund 5 days after injection, yet showed intermittent tremors, especially on stimulation. Even at doses of 100 μg./g. a similar condition persisted for 4 days after injection (448).

During malathion poisoning in insects, ChE is inhibited, as invertebrates. One investigator has reported an almost linear relationship between ChE inhibition and mortality (378), but much of the evidence shows a time course of inhibition difficult to reconcile with the march of hyperactivity and mortality, especially as it is usually assumed that ChE is present in great excess, some 90% having to be inhibited before the effects of acetylcholine accumulation are apparent (91,269). When house flies were poisoned with malathion, the whole-fly ChE was almost completely inhibited within a few hours, but this inhibition soon diminished and, by the time flies started to die, was about 55% (448). Similar results were obtained when inhibition was followed in more restricted sites. With a number of organophosphorus insecticides, including parathion, applied at LD_{50} doses to house flies, there was usually a fairly close association between paralytic symptoms and maximum head-ChE inhibition. With malathion, however, maximum ChE inhibition occurred during the period of hyperactivity, ChE activity having largely recovered before any flies were paralyzed (414). A similar reduction in inhibited ChE has been noted in the nerve cord of cockroaches poisoned with malathion (448). In flies poisoned with sure lethal doses of parathion and DFP the head-ChE was fully inhibited within an hour, and there was no recovery; with malathion there was a relatively slow increase in the proportion of head-ChE inhibited, and full inhibition was never quite achieved (542), although possibly sufficient ChE was inhibited to interfere with brain activity.

The active inhibitor is probably malaoxon (394,448,449), the oxygen analogue of malathion, which is several hundred to many thousand times more active than malathion against mammalian and insect cholinesterase (394,450). However, malaoxon could not be detected chromatographically in tissues of cockroaches previously injected with malathion, although other techniques suggested that malaoxon was in the products and made it clear that the inhibitor was not malathion (450).

Malathion, like the more potent inhibitor, DFP, causes an accumulation of free glutamine in the house fly thorax at the prostrate stage and also stimulates oxygen consumption despite paralysis of the insect. These results cannot readily be attributed to inhibition of ChE and suggest the occurrence of other biochemical disturbances. Further, although the lethal action of malathion in mammals may be reversed by injections of pyridine 2-aldoxime methiodide (PAM) and atropine, these materials have no effect on the course of poisoning in house flies, suggesting again that there is some biochemical lesion additional to ChE inhibition (24,642,643). Since it is not known whether PAM will reactivate insect ChE, it is perhaps worth noting that PAM is not equally effective on all ChE; with vertebrates the aged form of inhibited ChE cannot be reactivated by nucleophilic reagents (55,60).

In vitro malathion can also inhibit cytochrome oxidase (433) and succinoxidase (448), enzymes not directly connected with acetylcholine, but these effects have not been linked with its insecticidal action. Finally, in cockroaches and house flies the glycolytic and tricarboxylic acid cycle pathways are not substantially inhibited by malathion, and hence it is unlikely that interference with carbohydrate metabolism is involved in the insecticidal action (449; cf. 50).

In the cockroach, as in vertebrates, there are systems capable of both activating and degrading malathion. Activation occurs with foregut and hindgut but especially with midgut and fat body, while malaoxon (the probable inhibitor produced during activation) is unaffected by gut preparations, but is degraded by the fat body to a noninhibitor (450). But breakdown of malathion in cockroaches appears to be much less extensive and complex than in warm-blooded animals, the cockroach producing two compounds compared with seven in the hen and the mouse. As most malathion metabolites of warm-blooded animals are of a low order of toxicity, inability of the insect to form such metabolites may contribute in a major way to the effectiveness of malathion as an insecticide (394). This is well illustrated by the observations that, in mouse liver preparations, the initial rapid activation of added malathion is opposed by a vigorous degradation and after 15 minutes anticholinesterase activity decreases, while in live cockroaches or in cockroach gut preparations degradation is very slight and there is a steady increase in total inhibitor activity (450). There is no information on degradation of malathion by other insects, but the fact that some materials of low or negligible insecticidal activity potentiate the toxicity of malathion to house flies suggests the presence of detoxification mechanisms. However, experiments which readily demonstrate enzymic hydrolysis of malathion with mammalian liver preparations failed to demonstrate this mechanism in either susceptible or malathion-resistant house flies (619).

There is both laboratory and field evidence for synergistic and antagonistic effects when other materials are used in conjunction with malathion. During field trials toxaphene, dieldrin, and heptachlor were tested with and without addition of malathion for aphid control. Addition of malathion to dieldrin did not affect control, but addition of malathion to heptachlor depressed the average control from 85 to 76%. Addition of malathion to toxaphene increased the initial kill from 42 to 71% and the average control from 44 to 64% (426). But addition of malathion to toxaphene did not improve control of cotton thrips obtained with toxaphene alone (133). Neither malathion at 1 or 2 lb./acre nor Perthane* at 1 or 1.75 lb./acre gave satisfactory control of cabbage looper, *Trichoplusia ni*, but a malathion-perthane combination at 1 lb. of each material per acre gave outstandingly good control (56).

* Registered trademark Rohm & Haas Co., Philadelphia, Pa.

The effects obtained may differ at different temperatures. In laboratory tests with the boll weevil, *Anthonomus grandis,* toxaphene and malathion were synergistic at high temperatures but antagonistic at lower temperatures, endrin and malathion synergistic at the lower temperatures, slightly antagonistic at 90°F. (655).

A number of the known pyrethrum synergists effectively potentiate organophosphorus insecticides against house flies, increasing initial toxicity and extending the duration of effectiveness of residual deposits. But some which were effective synergists when tested with other organophosphorus insecticides did not potentiate the toxicity of malathion (284). Indeed, piperonyl butoxide antagonized malathion (482,484). This antagonism was found with both DDT-susceptible and DDT-resistant strains of house fly, the data suggesting that the antagonistic mechanism was similar in both strains at a number of temperatures (482). Antagonism was even more pronounced if piperonyl butoxide was applied a short time before application of malathion; as this interval increased, the antagonism of malathion toxicity diminished, but was still detectable when application of piperonyl butoxide preceded application of malathion by 30 hours (485).

The basis of the antagonism may be the decreased inhibition of ChE which occurs when malathion is used in combination with piperonyl butoxide, even though this is much less pronounced with house fly ChE *in vivo* than with bovine ChE *in vitro* (484). However, this could hardly explain the findings that although piperonyl butoxide antagonizes malathion to house flies and to the Madeira roach, *Leucophaea maderae,* it potentiates malathion to the German roach, *Blattella germanica* (118) and dermestid larvae (399).

Piperonyl butoxide potentiates the toxicity of some malathion analogues but antagonizes others; possibly this is associated with the length of the alkoxy grouping (613).

D. RESISTANCE TO MALATHION

Two (or possibly three) mites, house flies, a mosquito, and possibly an aphid are known to be markedly resistant to malathion.

Strains of *Panonychus ulmi* resistant to malathion appeared in several districts of British Columbia in 1953 (161).

A strain of *Tetranychus telarius,* isolated in 1949 and subsequently reared without contact with insecticide, was found to be highly resistant to malathion in 1955. When crossed with a nonresistant strain, segregation ratios indicated that, in this species, malathion resistance is dominant and probably involves a single factor (587).

Possibly *Tetranychus cinnabarinus* is, or is becoming, resistant to malathion in Arizona (216).

In California larvae and adults of the mosquito, *Culex tarsalis*, developed malathion resistance after 2.5 years use of malathion; resistance of larvae was 21–33, that of adults 93–100, times normal (219). Malathion resistance has not yet appeared in *C. tarsalis* in Oregon (172).

Malathion resistance in house flies, first detected in 1955 (369), has been recorded from Arizona, Florida, Georgia, and New Jersey. Development of malathion resistance may be somewhat erratic, populations at some farms remaining susceptible while others in the district have definite resistance. Further, resistance may be lost, fly populations resistant to malathion baits in the late summer losing that resistance during the winter, effective control being obtained with the same bait the following year (26).

In central Florida adult house flies from a poultry house where malathion had been used as a larvacide, and as both residual and bait sprays for 2.5 years, were about 40-fold resistant to malathion sprays and rather slighly resistant to Dipterex,* ronnel, and parathion; after 20 generations of selection this strain become 100-fold resistant (641). This strain was also resistant to malathion in baits, the LC_{50} being more than 100 times normal. Part of this difference represents behavioral resistance, since bait tests involve acceptance as well as toxicity of the insecticide (346), many surviving flies showing extreme avoidance of malathion bait (525). Malathion resistance appears widespread in Florida, flies from eight dairies and six poultry houses being 3–133 times resistant to malathion and often resistant to Dipterex, Diazinon, and parathion (347).

Following upon 3 years of excellent house fly control with malathion sprays and baits, resistance appeared in one dairy barn in Georgia. As in Florida, resistance was in part behavioristic, flies approaching but not alighting on the malathion baits, in part physiological, the LD_{50} of topically applied malathion being 1.2 μg. per fly compared with 0.5 μg. for a nonresistant strain (331). Another strain from Georgia, initially about 2-fold resistant to topically applied malathion, became some 14 times resistant on further selection with residual deposits; concurrently there was an increase in behavioristic resistance, the decrease in number of flies visiting baits being accompanied by greatly increased survival (181).

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* Registered trademark Chemagro Corp., Kansas City, Mo.

When colonized without further selection, a Georgia strain was about 20 times resistant to topically applied malathion, an Arizona strain somewhat less resistant (526).

Cross resistance from other insecticides to malathion is usually absent, the following resistant strains being fully susceptible to malathion: DDT$_r$ *Pediculus humanus humanus* (100), chlordane$_r$ *Blattella germanica* (658), endrin$_r$ *Anthonomus grandis* (655), dieldrin$_r$ *Anopheles quadrimaculatus* (402), parathion$_r$ *Aedes nigromaculata* (359), and the blue tick, *Boophilus decoloratus*, which is resistant to arsenic and Group II insecticides (627). However, Diazinon$_r$ strains of house flies from New Jersey were resistant to malathion (185,248).

Strains of the alfalfa aphid, *Therioaphis maculata,* resistant to parathion, have appeared in California (568); malathion is sometimes effective, sometimes ineffective, against these resistant strains (570).

Two malathion$_r$ strains of house fly were normally susceptible to synergized pyrethrins (78), two other strains 8–10-fold resistant to two carbamate insecticides (348).

E. PHYTOTOXICITY

Most formulations and concentrations of malathion are noninjurious to plants, although various exceptions have been noted. Poinsettias were severely damaged at concentrations as low as 0.05% (265). There are also reports of injury to Boston fern and to maize in the whorl stage, of stunting of cucumbers in the runner stage (240), and of injury to olive fruits (560). Malathion formulations were considerably phytotoxic to a variety of shade and ornamental trees. Most were severely damaged by 3% emulsion, and some were killed outright by 5% sprays. Species showing pronounced damage were *Ulmus americana, Crataegus cordata, Populus* sp., *Salix* sp., *Quercus palustris, Carya armata,* and *Virburnum lantana* (96). Offsetting these observations is the fact that malathion has been used on a wide variety of plants without obvious damage.

Malathion is not normally used as a seed dressing or as a soil insecticide. There are, therefore, few references to damage or otherwise to seeds and root systems. Germination of flower seeds was not affected after 6 months storage with malathion dust (577). Emergence was little affected when onions were furrow sown with malathion (293). However, 1.125 oz. of malathion per bushel of soybean seed caused a 37% drop in emergence and a significant increase in mean emergence period (564). Likewise, wettable powder drilled with beet seeds at 1 and 3 lb. of malathion per acre reduced germination 32%, and final beets were smaller than those of untreated plants; even 0.5 lb. caused a drastic reduction in germination and in final yield (225,226). Malathion was noninjurious to pine seedling root growth (540).

F. STABILITY AND COMPATIBILITY

Malathion is a reasonably stable compound, but undergoes some decomposition when held at temperatures much above room temperature. Decomposition of both malathion and liquid formulations occurs when these are stored for long periods in the presence of iron. Malathion is readily hydrolyzed in highly alkaline or mildly acid media. At pH 12 hydrolysis was almost instantaneous; at pH 9, 50% was hydrolyzed in about 12 hours; but no hydrolysis occurred over 12 days at pH 5–7 (652).

Emulsifiable concentrates with nonionic materials may be expected to have long shelf life; certain anionic emulsifiers may be less satisfactory (652).

Dilute emulsions are stable over long periods.

Although some carriers markedly accelerate the rate of malathion breakdown, stable wettable powder preparations are possible with proper choice of carrier. In general a slightly acidic or neutral reaction with pH 4–7 and a low moisture content are desirable characteristics for inerts used in preparation of malathion wettable powders. Carriers giving the most stable formulations include acidic kaolin, acidic frianites, and diatomaceous earths (652).

Long-term stability of dilute dusts can be achieved with proper choice of carrier; pyrophyllites, neutral talcs, gypsum, and even some calcium carbonates appear to be satisfactory, malathion being surprisingly stable on a treated calcium carbonate, even though the carbonate was somewhat basic in reaction (652).

More recently it has been shown that the loss with some carrier dusts may exceed half the malathion in 20 days of storage at 40°C. The surface acidity (pK_a) of the carrier is of prime importance for stability of malathion dust formulations, and, in general, a pK_a of more than 3 is a necessity if a malathion dust is to be stable. With some carriers loss of malathion can be remarkably reduced and sometimes quite eliminated by addition of small amounts of stabilizers, namely, 0.7% of polyoxyethylene alkyl ether (PAE) or 0.5% PAE plus 0.5% fatty acid, but these stabilizers were not effective when acid clay was the diluent (405,406).

Surface acidity is probably not the only factor promoting malathion breakdown in dusts. Those mineral carriers with a positive alcoholic adsorption index—acid clays, attaclay, bentonite, and acid-treated clays—promoted rapid breakdown of malathion in dust formulations and were not stabilized by addition of nonionic surface active materials. Carriers with a negative alcoholic adsorption index—kieselguhr, clay, talc, and silica sand—were effectively stabilized toward malathion by polyoxyethylene lauryl ether, fatty acids, or mixtures of these. These materials appear to be tightly adsorbed on the carrier and so isolate malathion from the carrier surface (403,404).

When polyoxyethylene lauryl ethers and polyoxyethylene alkyl phenyl ethers of varying polymerizations ($\bar{P}$) were tested, those with $\bar{P} = 7$–10 were superior as malathion stabilizers. The optimum quantity was 0.7% (404).

Long-term compatibility of malathion with fungicides and insecticides may vary from good to poor, and some malathion-fungicide mixes would, if they were prepared, have short shelf life, soluble coppers, metallic salts of dithiocarbamic acid (ferbam and maneb), and some wettable sulfurs in particular inducing fairly rapid breakdown. Malathion has reasonable long-term stability with most chlorinated hydrocarbon insecticides, except toxaphene. Neutral calcium arsenate caused fairly rapid breakdown of malathion during storage (652).

Although alkaline substances and some metallic ions catalyze or induce decomposition of malathion in formulations, the insecticide can be tank-mixed and used successfully with most commercial fungicides including soluble coppers, zineb, ferbam, etc., as well as with mildly alkaline substances (652). Malathion is not compatible with hydrated lime or bordeaux mixture (34).

G. PERSISTENCE

On inert substrates, in the laboratory, malathion deposits remain insecticidal for weeks and even months. On plants, in the field, the effective life is seldom more than a few days. On plants, indoors, malathion may be more persistent, residual toxicity on rice plants kept indoors being twice that on plants in the field (300). Yet this only partially explains the few cases when malathion applied to plants has shown considerable persistence with effective control for relatively long periods (77,341,382,472,504).

Effect of climatic factors on persistence has not been fully investigated. In laboratory tests, a 24 hour delay reduced toxicity to cotton leaf worm by 43% (429). In field cage tests, toxicity to boll weevil dropped 38% during the first 24 hours; in laboratory tests the loss was 74% with boll weevil, 99% with cotton leaf worm (208). Yet in similar laboratory studies a delay of 24 hours reduced toxicity to boll weevil by only 6%; a similar delay plus a 5 m.p.h. wind reduced toxicity by only 10%, but a 24 hour delay plus 0.5 inch of rain reduced toxicity by 76% (276).

When malathion was deposited on tempered masonite panels, aging the deposit for 21 or 60 days did not greatly affect toxicity to *Blattella germanica* (302). When insecticide deposits were exposed continually to an air current in the laboratory, only malathion, parathion, EPN, dieldrin, and DDT were effective after 1 week, malathion deposits giving the most rapid knockdown of *Drosophila melanogaster*. The malathion deposit remained highly effective for another week and was still insecticidal after 5 weeks of weathering (169). Ply panels sprayed with 100 mg. of malathion per square foot have remained effective against *Anopheles quadrimaculatus* for 15–17 months (26,345). However, very light deposits on glass had lost toxicity to adult Japanese beetles after 4 and 9 days (212). Malathion deposited on woolen cloth caused complete kill of bed bugs 28 weeks after the cloth was treated, thus surpassing in persistence DDT, chlordane, lindane, Chlorthion, Diazinon, Dipterex,* and ronnel. Only dieldrin and pyrethrins + piperonyl butoxide were as persistent as malathion (374).

In some circumstances, malathion-treated soil may remain toxic for long periods (171).

* Registered trademark Chemagro Corp., Kansas City, Mo.

H. RESIDUES ON PLANTS

As with other insecticides, deposits of malathion decrease in an exponential manner with regard to time (647). Hence deposits may be conveniently characterized by their half-lives. Often there are characteristically two half-lives, the shorter measuring the initial rapid dissipation of insecticide while it remains on the surface subject to physical and chemical diminution by weathering, the second and longer half-life resulting from penetration of insecticide into the plant tissue where decrease in concentration is principally due to metabolic attack and to increase in plant size.

For Valencia oranges sprayed with 3 lb. of malathion wettable powder per 100 gal., the degradation half-life was 7 days and the persistent half-life 32 days (63).

Usually, however, no such distinction is made in the results reported, perhaps because, in general, deposits of malathion are so rapidly lost from fruit and vegetables (data are summarized in Table III) that there is seldom any suggestion of a residue problem. The usual recommendation is to allow a minimum of 3 days between the last application and harvest for field-grown crops and 7 days between last application and harvest for crops under glass. In many cases, even the initial deposits on fruit (cf. apples, pears, peaches, and oranges, Table III) are below the recommended acceptance level (626). Further, the ordinary washing of vegetables as done by the housewife in preparing them for the table usually reduces malathion residues to less than 1 p.p.m. (551). Washing and blanching for canning or quick freezing have the same effect (151,237,610), and the amount in the final product is negligible (427). There are also considerable losses on dehydration. Alfalfa sprayed with 15 oz. of malathion per acre assayed 62 p.p.m. of malathion when harvested and chopped green, 19 p.p.m. after dehydration (483).

Table III contains the data on residues published prior or additional to that of Wallis *et al.* (610). That paper contains an extensive tabulation of the residues remaining on vegetables, berries, and tobacco crops at various times after application of 1.7 lb. of malathion per acre and should be consulted in conjunction with Table III (cf. 72).

TABLE III

Amounts of Malathion on Plants at Various Times after Application

Crop	Dose per application	Formulation	Malathion residue, p.p.m.	Time after last application	Ref.
	lb./acre				
Alfalfa	1.0	Emulsion	46.6	1 day	154
			8.1	5 days	154
			0.8	10 days	154
			0.4	15 days	154
	1.17	Dust	2.0	1 day	154
			0.8	5 days	154
			0.3	10 days	154
			0.2	15 days	154
Artichoke	1.8	Dust	<0.2	2 days	178
			0	8 days	178
			0	15 days	178
Asparagus	1.7	Emulsion	4.49	0 day	151
			Trace	1 day	151
			0	3 days	151
			0	7 days	151
Beans (lima)	0.8	Dust	74.0	1 hour	218
			18.0	1 day	218
			0	2 days	218
	1.6	Dust	94.4	1 hour	218
			22.7	1 day	218
			1.0	2 days	218
Beans (snap)	3.75	Dust	0.2	0 day	626
			0	7 days	626
			0	12 days	626
Broccoli	2.5	Dust	9.2	0 day	626
			3.7	1 day	626
			0	6 days	626
Cabbage	0.63	Emulsion	14.7	4 hours	605
			13.3	1 day	605
			8.6	2 days	605
			2.4	7 days	605
	1.04	Emulsion	41.3	4 hours	605
			31.6	1 day	605
			10.8	3 days	605
			0.1	7 days	605
	1.25	Emulsion	45.7	4 hours	605
			30.4	1 day	605
			28.6	2 days	605
			8.7	7 days	605

(continued)

TABLE III (*continued*)

Crop	Dose per application	Formulation	Malathion residue, p.p.m.	Time after last application	Ref.
Cabbage (*cont.*)	1.9	Emulsion	91.3	4 hours	605
			72.0	1 day	605
			55.0	2 days	605
			24.8	7 days	605
			24.8	7 days	605
	2.6	Dust	4.2–9.5	0 day	178
			0.4–0.5	5 days	178
Collards	0.5	Emulsion	0.7–1.7	3 days	606
	1.0		2.6–3.9	3 days	606
			1.1–1.9	7 days	606
Cucumber	7.75	Dust	0.3	0 day	626
			Trace	7 days	626
			0	12 days	626
Melon	19–28	Dust	<0.2	24 days	178
Onion (foliage)	0.5	Wettable powder	17.0	1 day	550
			0	3 days	550
			18.8	1 hour	550
			13.6	1 day	550
			3.3	2 days	550
			2.1	3 days	550
			0.9	6 days	550
			0.2	7 days	550
	1.0	Emulsion	7.2	1 day	550
			0	3 days	550
			10.6	1 hour	550
			4.0	1 day	550
			1.7	2 days	550
			1 0	3 days	550
			0.5	6 days	550
			0.2	7 days	550
	1.2	Dust	3.7	1 day	550
			0	3 days	550
			3.6	1 hour	550
			4.1	1 day	550
			2.5	2 days	550
			1.6	3 days	550
			0.4	6 days	550
			0	7 days	550
Spinach	0.75	Wettable powder	12–14	1 day	237
			0.3–0.5	6 days	237
	1.25	Emulsion	80.5	4 hours	598

TABLE III (*continued*)

Crop	Dose per application	Formulation	Malathion residue, p.p.m.	Time after last application	Ref.
			13.0	3 days	598
			2.2	7 days	598
	1.9	Emulsion	142.8	4 hours	598
			29.5	3 days	598
			6.2	7 days	598
	2.3	Dust	101	0 hour	572
			49	1 day	572
			0.4	5 days	572
			101	0 hour	572
			49	1 day	572
			8.1	5 days	572
	3.2	Dust	167	0 hour	572
			89	1 day	572
			0.35	5 days	572
	9.45	Dust	60.9	0 day	626
			3.9	7 days	626
			Trace	12 days	626
Turnip (tops)	0.63	Emulsion	1.3–1.5	4 hours	606
			1.0–1.2	1 day	606
			0.8–1.1	2 days	606
			0.6–0.9	7 days	606
	1.25	Emulsion	1.8–2.3	4 hours	606
			1.7–2.2	1 day	606
			1.0–1.7	2 days	606
			0.9–1.1	7 days	606
	1.9	Emulsion	4.1–5.0	4 hours	606
			3.2–3.8	1 day	606
			2.5–3.1	2 days	606
	lb./100 gal.				
Apple	0.31	Emulsion	1.0	0 day	626
			0.1	7 days	626
			Trace	14 days	626
	0.31	Wettable powder	7.4	0 day	626
			0.1	7 days	626
			Trace	14 days	626
			1.8	0 day	626
			1.3	4 days	626
			0.9	6 days	626
			0.2	10 days	626
	0.50	Emulsion	1.2	0 day	626

(*continued*)

TABLE III (*continued*)

Crop	Dose per application	Formulation	Malathion residue, p.p.m.	Time after last application	Ref.
Apple (*cont*)			0.1	7 days	626
			Trace	14 days	626
			1.0	0 day	626
			0.3	3 days	626
			0.2	5 days	626
			0	12 days	626
	0.50	Wettable powder	6.6	0 day	626
			0.3	7 days	626
			Trace	14 days	626
			1.6	0 day	626
			0.6	3 days	626
			0.4	5 days	626
			Trace	12 days	626
	0.62	Emulsion	3.1	0 day	626
			0.2	7 days	626
			Trace	14 days	626
	0.62	Emulsion	5.2	0 day	626
			0.8	4 days	626
			0.4	6 days	626
			0.2	10 days	626
	0.75	Wettable powder	21.1	0 day	626
			1.0	7 days	626
			0.3	14 days	626
	0.75	Wettable powder	5.7	0 day	626
			2.3	3 days	626
			1.7	5 days	626
			Trace	12 days	626
	1.25	Emulsion	8.1	0 day	626
			0.6	7 days	626
			0.3	14 days	626
	1.25	Emulsion	3.7	0 day	626
			1.7	3 days	626
			1.1	5 days	626
			Trace	12 days	626
Citrus (peel)	0.75		3.5	1 day	63
			2.9	2 days	63
			1.5	7 days	63
			1.0	14 days	63
			0.5	21 days	63
			0.4	28 days	63
			0.2	62 days	63

TABLE III (*continued*)

Crop	Dose per application	Formulation	Malathion residue, p.p.m.	Time after last application	Ref.
Pears	0.31 0.5 0.62 0.93	Emulsions and wettable powders	Initial and all subsequent deposits <5.0		626
Peaches	0.31	Emulsion	6.0	0 day	626
			2.3	7 days	626
			1.2	14 days	626
			Trace	41 days	626
	0.62	Emulsion	12.8	0 day	626
			2.5	7 days	626
			1.2	14 days	626
			Trace	37 days	626
	0.62	Wettable powder	18.8	0 day	626
			4.6	7 days	626
			1.3	14 days	626
			Trace	41 days	626
	g./1000 cu. ft.				
Lettuce	1.0	Aerosol	9.75	1 day	550
			3.46	3 days	550
			0.85	6 days	550
			0.46	9 days	550
			0	12 days	550
	1.5	Aerosol	21.9; 21.2	1 hour	550
			15.3; 15.9	1 day	550
			20.5; 12.4	3 days	550
			3.6; 3.8	6 days	550
			0.4; 0.3	9 days	550
			0	13 days	550
Tomato (fruit)	1.0	Aerosol	0.31–0.41	1 hour	550
			0.32–0.90	1 day	550
			0	3 days	550
			0	6 days	550
			0	9 days	550
			0	12 days	550
	1.5	Aerosol	1.05	1 hour	550
			0.60	1 day	550
			0.26	3 days	550
			0.14	6 days	550
			0.02	9 days	550
			0	12 days	550

I. RESIDUES IN STORED PRODUCTS

On wheat in storage, malathion residues had an average half-life of 5.6 months (234). However, the gross malathion residue does not appear in the processed product. Milling tests have shown that 0.0 to less than 0.3 p.p.m. of malathion occurred in flour derived from wheat dusted or sprayed with malathion at dosages of from 2.5 to 7.5 p.p.m. Shorts, bran, and germ contained more malathion, but the amount did not exceed 3.4 p.p.m., even when wheat was milled a few days after insecticide was applied (524).

J. RESIDUES IN ANIMAL PRODUCTS

Malathion does not accumulate in the fat of cattle sprayed repeatedly with malathion (95). No detectable malathion was found in cuts of foreleg, hindleg, and rump of heifers sacrificed 2 weeks after application of 0.5% P^{32}-malathion emulsion spray, but water-soluble metabolites were detected in meat cuts and in thymus, thyroid, pancreas, liver, and bone to the extent of 0.05–2.0 p.p.m. (395). Pigs sacrificed 3 days after being sprayed twice within 7 days with 1% malathion emulsion contained no malathion in the fat or in lean meat and either no malathion or a trace (0.04 p.p.m.) in the liver (309). Similar results have been obtained with hens on a diet containing 100 p.p.m. of P^{32}-malathion. Breast and drumstick contained a maximum of 1.18 and 0.5 p.p.m. of malathion or metabolites during feeding, almost all of which was eliminated within 10 days on normal feed. The liver, gizzard, kidney, and blood also contained malathion or metabolites during feeding, a maximum amount of 2.65 p.p.m. being found in the kidney, which was presumably concentrating it during excretion. The amounts soon became insignificant when hens were returned to a normal diet (394). When dairy cows were sprayed or dusted with malathion, insecticide was present in milk samples taken 5 hours after treatment but did not exceed 0.64 p.p.m.; traces were sometimes present at 12 and 24 hours, but at 3 and 7 days the milk was virtually free of insecticide (95,231,621). Eggs may contain up to 0.10 p.p.m. of malathion or metabolites when hens are fed a diet containing 100 p.p.m. of P^{32}-malathion. Residues in eggs from sprayed hens were about $^1/_{10}$ as much (394).

K. RESIDUES CAUSING TAINT

The flavor of eggs was not affected when hens were fed a diet containing 50 p.p.m. of malathion (197,602), and there were no adverse effects on flavor of milk from cows fed malathion-treated alfalfa (221).

Malathion treatments had no effect on the flavor of beans (73,150), peas (187), lettuce (290), asparagus (151), grapes, melons, and figs (279), or canned tomatoes (57). A difference in flavor, not necessarily an off flavor, has been reported for malathion-treated apples, pears, beans, and peas (253). Strong flavors have also been detected in apple sauce and tinned pears from malathion-treated fruit, but as the effects were not reproducible, other factors may have been responsible (279). One case of definite off flavor in the unacceptable category has been reported in peaches following use of malathion sprays, even though 83 days had elapsed between the last spray and harvest (16,506). This may, however, be an isolated case associated with early experimental samples of malathion, for malathion is now widely used in peach orchards for control of green peach aphid (30). In England malathion has not produced taint when used at recommended rates (98).

Milling and baking tests on wheat treated with 4 and 8 p.p.m. of malathion indicated no adverse effects on quality, and no foreign odor was detected during baking or in cut loaves (11,615).

The general conclusion is that malathion can be used without fear of producing off flavors in the marketable product.

II. Use of Malathion for Insect and Mite Control

A. VETERINARY, MEDICAL, AND PUBLIC HEALTH PESTS

The combination of high toxicity to insects and low acute and chronic toxicity to vertebrates possessed by malathion immediately suggests its use on livestock for control of ectoparasites. It is now widely used for this purpose, and possibly this is where it will ultimately find its best application. Malathion is not a systemic insecticide in mammals (3).

1. *Acarina*

a. Ticks. Malathion has been found effective against *Argas persicae*, the fowl tick (509,510), provided the ticks came in contact with insecticide for 9–34 hours, ineffective if the building contained numerous hiding places into which insecticide did not penetrate (198). Malathion was ineffective against one ixodid tick, *Rhipicephalus sanguineus*, the brown dog tick (247), and only moderately effective against another, *Boophilus microplus*, the cattle tick (355).

b. Mites. Very effective control of northern fowl mite, *Ornithonyssus sylvarium;* chicken mite, *Dermanyssus gallinae;* hog mange mite, *Sarcoptes scabaei suis;* myocoptic mange mite, *Myocoptes musculinus;* and a scab mite, *Myobia musculini*, has been obtained with malathion.

Northern fowl mite—Treating litter with 4% malathion dust at from 10 to 50 lb. per 1000 square feet plus a handful of dust in each nesting box give immediate and effective control. To control heavy infestations it was necessary to dust birds individually, using about 5 g. of 4% dust, or to spray the flock with an 0.5% emulsion, 1 gal. to 100 birds. All treatments gave about 4–6 weeks protection against reinfestation, although under natural conditions infestations may not occur for up to 12 weeks (196,197,256,282,602). A light infestation was eliminated from flocks in community-type cages by spraying woodwork and wire with 0.5% malathion emulsion (512). Dusting infected individuals as detected, rather than the whole flock routinely, gave both effective and economical control (513). However, failures to obtain eradication have been reported (494), and malathion is said to be ineffective in Georgia, Alabama, and Michigan (371).

Chicken mite—Spraying roosts, walls, ceilings, and other likely harborages with 1 or 3% malathion or treating litter with 4% dust at 50 lb. per 1000 square feet gave excellent or complete control, in some cases for more than 45 days (197,198,430,492). Dusting the wire floor of individual cages with 4% malathion or spraying woodwork and wire of community-type cages with 0.5% malathion gave complete control for more than 1 or 2 months (512). In Japan thorough spraying of batteries, without removing the hens, with 0.025% malathion almost eradicated chicken mite, and few were discovered for at least 20 days after application. Higher concentrations did not give conspicuously better control. Infestations were easily controlled by repeated applications (334).

Hog mange mite—Spraying with 0.5 or 1% malathion emulsion, about 1 qt. per animal, completely eliminated a heavy infestation (493).

Myocoptic mange mite; scab mite—A heavy infestation of *Myo-*

coptes musculinus on laboratory mice was completely controlled by dipping the mice in a 2% malathion emulsion (134). Heavy combined infestations of *M. musculinus* and *Myobia musculini* were readily controlled by application of malathion dust to bedding in the animal jars (618).

Feather mites—A heavy mite infestation on caged parakeets and cockateels was readily controlled by putting malathion dust in each nest box (121).

Chiggers—Malathion at 0.5–2 lb. per acre gave 1 or 2 weeks of good control of *Eutrombicula alfreddugesi,* but was much inferior to toxaphene and dieldrin (320).

2. Insecta

a. Anoplura. *Haematopinus suis* (hog louse)—One spray of 1% malathion emulsion gave protection for more than 30 days (309).

Haematopinus eurysternus, Solenopotes capillatus, Linognathus vituli (cattle lice)—Malathion 0.05 and 0.5% gave a complete kill of *H. eurysternus* and remained effective 1 week and 2 weeks, respectively; other insecticides were equally effective (545). In other tests 0.5% sprays gave better than 130 days of satisfactory control (32,139). Complete control of all the above lice was obtained when cattle were allowed access to self-rubbing devices treated with malathion 2 or 5% in fuel oil (233).

Pediculus humanus humanus (body louse)—DDT$_{\mathrm{r}}$ lice are susceptible to malathion; in limited sleeve tests with humans a 1% malathion dust remained fully effective for almost 3 weeks (100,101).

b. Mallophaga. Malathion has proved most effective against all species of biting lice as yet tested.

Bovicola bovis (cattle louse)—Complete control was obtained when cattle were allowed access to self-rubbing devices treated with malathion 2 or 5% in fuel oil (233).

Bovicola caprae, B. limbatus (goat lice)—Dips containing 0.025–0.25% malathion gave complete control and prevented reinfestation for 4 weeks (545).

Menocanthus stramineus; Menopon gallinae; Goniocotes gallinae; Goniocotes hologaster (chicken lice)—Spraying roosts with 3% malathion gave effective control of *M. stramineus* for from 3 to 5 weeks (198).

Treating each bird with 6 g. of 1% dust gave complete control of *M. stramineus*, but reinfestation occurred within 2–4 weeks (545).

Treating litter with malathion 4% dust at 5–50 lb. per 1000 square feet, plus a handful in each nest, gave complete initial control of *M. stramineus;* at the higher doses flocks remained clean for 5–10 weeks (258,511). Treatment of the whole litter area was unnecessary, for more than 6 weeks control was obtained when 4 lb. of 4% malathion per 100 birds was applied to the dust wallows and corners only. As a further refinement, more than 35 days complete control was obtained when hens carried out self-treatment in dust bath boxes containing 1 lb. of 4% malathion per 100 birds (511).

Treating litter with 4% dust at 6.7 lb. per 1000 square feet gave more than 21 days complete control of *M. stramineus* and *M. gallinae* (282). Spraying the roosting area once with a 3% malathion emulsion gave better than 8 weeks control of *M. stramineus, M. gallinae,* and *G. hologaster* (430). Spraying walls, ceilings, roosts, and nests with 1% malathion gave more than 45 days complete control of *M. stramineus, M. gallinae,* and *G. gallinae,* provided hens were not excluded from the house during spraying. Malathion 4% dust applied to litter at 50 lb. per 1000 square feet also gave more than 45 days complete control of these species (492); at half that dose of active malathion, fowls remained louse-free for at least 8 weeks (494).

Spraying range turkeys with 1% malathion gave complete control, the flocks remaining louse-free as long as 3 weeks; a 0.5% spray was only partially effective (224).

Lipeurus baculus; Goniocotes bidentatus (pigeon lice)—Dusting pigeons individually with 0.5 or 1.0% malathion and treating nests with 1% dust both gave better than 120 days control (514).

Lipeurus sp.; *Goniocotes* sp.; *Goniodes* sp.—Lice on partridge, quail, and pheasant were readily controlled by adding malathion dust to the natural dust wallows (515).

c. Hemiptera. *Cimex lectularius* (bed bug)—Spraying bed frames, mattresses, and woodwork with malathion 1.0 or 0.5% in odorless kerosene gave complete control (374).

d. Diptera. Mosquitoes—Breeding of *Culex* spp., principally *C. pipiens* and *C. stigmatosoma,* in log ponds was readily prevented by use of malathion; duration of control depended upon dose, 2.5 weeks at 1 lb. per acre, 6 weeks at 6 lb. per acre (360). Malathion has been used for control of culicine larvae that have become highly resistant to chlorinated hydrocarbons (318), but malathion resistance has emerged, larvae and adults of *C. tarsalis* developing malathion resistance after 2.5 years use of malathion (219). But malathion was not effective against DDT_r *C. pipens molestus* breeding in septic tanks (517). Against DDT_r strains of the salt-marsh mosquitoes, *Aedes taeniorhynchus* and *Aedes sollicitans*, malathion was effective at 0.05 and 0.1 lb. per acre which is about $^1/_5$ the effectiveness of parathion (200,317). Aerial application at about 0.25–0.5 lb. of malathion per acre continues to give effective control in southeastern States (26). Malathion is also effective against *A. nigromaculis* (220).

In field tests with thermal aerosols at 0.3 lb. per acre, the kill of *A. taeniorhynchus* averaged 92% at 330 feet and 87% at 660 feet, there being little variation in the kill obtained in widely separated areas in Florida; comparable kills with DDT were 28 and 26% (516). Comparison of malathion fog, dust, and spray treatments indicated that fogs were somewhat more effective than dusts; both usually gave appreciable control at the maximum distance of 810 feet (26).

In laboratory trials using *Anopheles quadrimaculatus* larvae, malathion had about $^1/_{10}$ the effectiveness of parathion. In field trials against *A. quadrimaculatus* and *A. crucians* malathion was reasonably effective at 0.05 lb. per acre, which is about $^1/_{50}$ as effective as parathion (318). Residual sprays at 100 and 200 mg. per square foot produced essentially 100% kill of $dieldrin_r$ *A. quadrimaculatus* for a year (26,402). For free-flying susceptible females the kill averaged 95–100% for 15–17 months. In occupied houses and farm buildings, deposits of 100 (26) or 200 (201) mg. achieved complete or almost complete kill during 4 months observation period.

Culicoides impunctatus (bloodsucking midge)—Surface application of 50 mg. per square foot reduced larval density to half, but had no long-term effect and did not constitute a practical control measure (327,328).

Culicoides furens (salt-marsh sandfly)—1 p.p.m. gave complete kill of larvae (547).

Tendipes plumosus—This midge, which sometimes occurs in plague numbers, is very susceptible to malathion, laboratory tests indicating 99% kill at 0.1 lb. per acre (278).

Simulium spp. (sandflies or blackflies)—In laboratory tests malathion was not effective against eggs or pupae of *Simulium venustum*

(281). Against *S. downsi* larvae malathion gave 95% kill at 10 p.p.m. and 77% at 1 p.p.m., but was less effective than some other insecticides (354).

Musca domestica (house fly)—Malathion has been tested as residual deposit, as bait spray, as scatter bait, and as larvacide against house flies; it has proved moderately but not outstandingly effective.

Over-all residual deposits from sprays containing 8–12 lb. of malathion per 100 gal. remained effective for 30 days (333,621). Deposits of 200 mg. per square foot gave immediate control, but this was not maintained beyond 7 (202) or 12 (316) days; essentially similar results were obtained with 100 mg. per square foot (202,329); residues of 50 mg. per square foot were not effective after 2 days (283). In Italy residues of 40 mg. per square foot began to lose effectiveness after the second week and were almost ineffective a week later (434). But duration of effectiveness may be acutely affected by the surface to which malathion is applied. On painted surfaces effectiveness was lost after 1 week, whereas deposits on unpainted surfaces remained effective for 8 weeks (329).

Sugar or molasses is sometimes added to malathion residual sprays, but there is little difference in effectiveness between sweetened and unsweetened sprays (283,310). In dairy barns, sprays containing 5 lb. of active insecticide per 100 gal. with or without sugar, gave 20–30 days protection (310); sprays containing 10 lb. of active insecticide + 20 lb. of sugar per 100 gal. gave 15–42 days control (246) and were not inferior to methoxychlor, lindane, or Chlorthion (249). Over-all sprays containing 12.5 lb. of active insecticide + 20 lb. of molasses per 100 gal. gave control for 3–4 weeks (230), although essentially the same spray (12.5 lb. of active insecticide + 20 lb. of sugar per 100 gal.) gave control for only 1–2 weeks (138). Residual bait sprays applied at 200 mg. of malathion + 500 mg. of sugar per square foot gave 7–8 weeks satisfactory control in some dairies, but failed to provide adequate control at other sites (329,330).

In the laboratory, both initial toxicity and duration of effectiveness of malathion deposits can be greatly increased by addition of a number of synergists (284), but the field possibilities of this approach seem not to have been explored. Here it may also be noted that residual effectiveness of some relatively volatile insecticides (lindane, aldrin, DDVP, and others) can be much extended by addition of nontoxic, nonvolatile materials such as chlorinated terphenyls. Such materials mask the bulk of the deposit from evaporation, but maintain a constant supply of fresh toxicant at the surface by diffusion from the body of the deposit. However, malathion has too low a vapor pressure for this technique to be spectacularly successful, effectiveness of deposits being improved from 4–5 to 10 days by addition of an equal amount of chlorinated terphenyl. When the amount of chlorinated terphenyl was increased to 4 times the amount of malathion, the deposit was less effective than that of malathion alone (291).

If a residual insecticide is not a practical method of control unless it gives at least 4 weeks of control (230), then, on average, residual deposits of malathion do not offer practical control of house flies.

Baits containing malathion and sugar or molasses appear to be more effective than residual deposits, although sometimes adequate control has not been achieved (249). Baits are applied frequently, often every day or second day, although the period of effectiveness of a single application may vary from 1 to 14 days depending upon bait concentration (333,353). Wet baits containing 1% malathion and 12.5% sugar gave outstandingly good control in dairy barns (353); those containing 2% malathion in molasses or malt were very effective for control of flies in a garbage dump (322). Much weaker bait may also be effective. Malathion 0.3% in 10% sugar gave effective fly control in dairies and poultry sheds (305). Five applications a week of bait containing 0.1% malathion in 10% molasses gave good to excellent fly control in open dairies; the same bait provided highly efficient fly control in a garbage dump where heavy breeding was occurring (204). In a similar situation, daily application of a malathion wettable powder was ineffective (24).

A dry scatter bait of sugar containing 1.0–0.5% malathion gave good control of flies in dairy barns and poultry sheds, when applied 4 days a week at about 100 g. of bait per 2500–5000 square feet. Heavier applications may be necessary at the beginning of treatment, but, once fly breeding has been reduced to a low level, normal rates of application maintain satisfactory control (203). Daily application of scatter bait containing 2% malathion and 10% granulated sugar in cornmeal was highly effective against flies in a substandard housing area and in dairies (321).

If neither bait sprays nor scatter baits are entirely satisfactory, control can be obtained by use of a large number of bait stations. These consist of wooden tongue depressors stapled to paper coasters or to pieces of screen, then coated with a slurry of sugar (and sometimes agar, sand, or other ingredients) containing 2% malathion; they remain effective for from 28 to 98 days (323).

Insecticide-treated strings or gauze strips have found some application in fly control. Without sugar, malathion was less effective than parathion or Diazinon (182); with sugar, malathion was equally as effective as Diazinon (49). Gauze strips heavily impregnated with malathion were still effective after 7 weeks of use, but a very long exposure was required for high kills (316).

Space sprays containing malathion were not particularly potent; in dairy barns good immediate control was achieved, but little or no control at 24 hours (640).

Malathion is effective against house fly larvae; 2 p.p.m. killed 40–60% of larvae (454); application of 0.1–3.6 g. of malathion per square foot gave either effective or complete control of larvae in fowl droppings, garbage piles, and turkey dung, but in this application malathion was not superior to other insecticides (143,285,335, 370,408,409,523,638).

When chicks were fed a single nontoxic dose of malathion, the feces voided during the first day were highly toxic to house fly larvae, but thereafter droppings were almost nontoxic (536).

Fannia sp. (lesser house fly)—Malathion (249) and malathion-DDVP (452) baits gave no control. Residual deposits are effective (26).

Callitroga macellaria and others including *Ophyra* spp. and *Sarcophaga* sp. (blowflies)—Baits containing 2% malathion in molasses or malt were very effective for control of blowflies on garbage dumps (322).

In national disaster or war it may be necessary to prevent fly infestation of the dead bodies of animals or men and to prevent further mutilation when infestation has already occurred. Malathion 0.5% spray protected fresh dog carcasses for 30 days, gave complete control of larvae in infested carcasses, and prevented reinfestation for 30 days, but was not superior to Diazinon, endrin, and dieldrin (44).

Lucilia cuprina (sheep blowfly)—In laboratory trials a malathion suspension containing 0.5% malathion prevented strike for 16 weeks compared with parathion 48.8 weeks and Diazinon 51 weeks (184). Malathion did not reduce the number of eggs laid on the fleece, but 0.25% sprays gave up to 10 weeks protection against larvae; Diazinon was greatly superior to malathion (505).

Siphona irritans (horn fly)—Almost perfect control of this pest was obtained when cattle were allowed access to self-rubbing devices treated with malathion, 5% in fuel oil (229). Residual applications of malathion to walls and surfaces of dairy barns did not reduce the numbers of horn flies on cows being brought into these barns for milking and feeding; Diazinon was effective for up to 7 weeks (593).

Hypoderma lineatum (warblefly)—A malathion 1% wash was quite ineffective (544).

Melophagus ovinus (sheep ked)—Malathion dust or spray gave effective control (464,519).

e. Siphonaptera. *Ctenocephalides felis* (cat flea); *C. canis* (dog flea)—A spray containing 1% malathion applied at 1 gal. per 1000 square feet of yard gave complete or almost complete control of these fleas for periods of up to 7 weeks (639). For flea control on cats (121) and dogs 5.0% dust or an 0.5% spray is acceptable; 0.25% dips are effective (26).

Echidnophaga gallinacea (sticktight flea)—Dusting litter with 13.5 lb. of 5% malathion dust per 1000 square feet did not eradicate a light infestation; with a second application at 50 lb. per 1000 square feet the infestation was eradicated (371).

B. AGRICULTURAL AND FORESTRY PESTS

1. Acarina

Penthaleus major (winter grain mite)—One application of malathion 0.25 lb. per acre gave excellent control (90).

Halotydeus destructor (red-legged earth mite)—Malathion at 0.5 oz. per acre (as used for control of lucerne flea) is partially effective; addition of DDT completes the control (607).

Tarsonemus pallidus (cyclamen mite)—On strawberries, malathion gave very poor control (597). This mite tends to build up following use of malathion (631).

Hemitarsonemus latus (broad mite)—Malathion gave poor control (538).

Brevipalpus inornatus—Malathion, 1.5 or 1 lb. per 100 gal., gave fair initial control, but was much inferior to other materials (241).

Petrobia apicalis (legume mite)—This mite is a serious pest of winter-grown legumes in Louisiana. Malathion at 2.8 lb. per 100 gal. gave complete control; 0.4 lb. per acre per application gave good control and is recommended for field use (543).

Petrobia latens (brown wheat mite)—Malathion, 1.5 lb. per acre, gave poor control (272).

Bryobia praetiosa (clover mite)—On apples, two applications of malathion, 0.25 and 0.5 lb. per 100 gal., gave perfect control until season's end, equalling parathion (135). One spray, 0.5 lb. per 100 gal., gave good initial control of summer generations, but the population soon increased (431). One application, between spring hatching and first oviposition, of 0.5 lb. of malathion per 100 gal. gave good control, but was inferior to demeton, parathion, and EPN (67).

In autumn and early spring this mite often invades houses in large numbers; malathion sprays or dusts applied to foundations, walls, and a strip around the house base will usually prevent these invasions (177,337,463), but one failure has been reported (162). In tenanted properties the objectionable odor of malathion may preclude its use (177).

Panonychus ulmi (European red mite)—Malathion is less toxic to winter eggs than to summer eggs (280,389), but this need not be emphasized for the major action is miticidal rather than ovicidal. In insectary tests 0.5 lb. per 100 gal. gave high mortality, but practically no kill was obtained at rates below 0.125 lb. per 100 gal. (34). One spray, pre- or postbloom, gave good control for 16 days. Summer control was much the same (362–364). Two sprays, 0.8 lb. per 100 gal., 3–4 weeks (135) or 5 days (390) apart gave near perfect control throughout the season. Concentrate sprays are also effective (38). Malathion has been reported as inferior to parathion against all stages (34,413). In some areas *P. ulmi* resistant to parathion are readily controlled by malathion (124), although there is evidence for slight cross tolerance (365), and strains occur resistant to both parathion and malathion (161).

Panonychus citri (citrus red mite)—Three sprays during the season, 0.3–1.5 lb. per 100 gal., gave commercial control of this species against which parathion is ineffective (559). Effective control was also obtained with malathion plus a special light oil, but the period of control was rather short (120). Mites 200–500 times field-resistant to demeton and highly cross tolerant to many organophosphorus insecticides were only 8 times resistant to malathion (306).

Oligonychus pratensis—On wheat, malathion at 2 lb. per acre gave good control at 5 days, but control was poor by 18 days (264).

Oligonychus ilicis (Southern red mite)—On *Ilex bullata*, 0.5 lb. of malathion per 100 gal. gave complete control at 3 and 8 days (241).

Oligonychus ununguis (spruce mite)—At about 0.25 lb. per 100 gal., malathion gave the usual picture of good initial control with reasonably rapid build-up after about 1 week. When the dose was 0.75 lb. per 100 gal., initial control was excellent, and there was no build-up even 29 days after spraying (437).

Eotetranychus hicoriae—Malathion was not effective for control of this mite on pecan (332). Population increases were limited following malathion application (614).

Tetranychus atlanticus—Malathion gave good control (148).

Tetranychus desertorum (desert spider mite)—In laboratory tests malathion had less than $^1/_{40}$ the toxicity of parathion to mites of this species (206).

There are conflicting reports on effectiveness in the field on cotton. In one trial a spray at 0.2 lb. per acre gave good initial kill, although build-up was occurring by the fourth day. A dust at 0.11 lb. per acre was somewhat more effective, yet at 0.27 lb. was almost ineffective (207). In another trial a spray at 0.25 lb. per 100 gal. gave poor kill and was quite ineffective beyond 5 days (415). Again, dust at 1 lb. and spray at 0.5 lb. per 100 gal. gave good immediate control, that of the dust extending beyond 8 days (508).

Tetranychus ludeni—Malathion dust was ineffective (633).

Tetranychus mcdanieli—A parathion$_r$ strain of this mite was perfectly controlled for 18 days with malathion at 0.4 lb. per 100 gal. (440). However, the block had been sprayed with parathion 9 days before the malathion was applied, and there is the possibility of joint action between applied malathion and parathion residue. That something of this nature may have occurred is suggested by later results when malathion at 0.5 lb. per 100 gal. gave good initial control, followed by very substantial build-up at 2 weeks (442).

Tetranychus marianae—Malathion gave good initial control (530).

Tetranychus pacificus (Pacific mite)—On apple, two sprays 3–4 weeks apart, 0.6 lb. per 100 gal., gave excellent control (135); at 0.25 lb. per 100 gal., malathion gave almost complete control of a parathion$_r$ strain (442).

Tetranychus telarius (two-spotted mite)—The green form is somewhat less susceptible to malathion than the red form (99).

Early experiments on apple and pear trees showed that malathion was effective for control of this mite (45); 0.25 lb. per 100 gal. gave very high mortality (34). In the laboratory malathion is more toxic than parathion to adults and larvae, but is much less effective against eggs. Moreover, it is but slightly translocated in the plant (170).

Hence, although the kill of adults and larvae may be complete, reinfestation occurs rapidly from mites which hatch after spraying (301). These observations set the pattern for malathion's behavior in the field where it usually gives good or sometimes perfect control for a few days followed by a population build-up from the many viable eggs (37,144,467,578,591). At doses of 0.5 lb. per 100 gal. or 1.4 lb. of dust per acre, good control is variously reported for 4–10 days (159,286,287,364,591). Occasionally, control may extend to 3 or more weeks (390).

However, five applications of 1.8 lb. per acre, as dust, gave poor control of *T. telarius* on strawberry (632), although four applications of 1.25 lb. per acre gave excellent control on potatoes where this mite builds up rapidly following use of DDT for flea-beetles and aphid control (351).

Heavy applications give fair control on beans (149,497,532,633).

A mixed spray comprising 0.45 lb. of the ovicide Ovotran + 1.25 lb. of malathion per 100 gal. has given exceptionally good control (144).

Aceria sheldoni (citrus bud mite)—Malathion not effective (356).

Aceria tulipae (wheat curl mite)—Malathion, 2 lb. per acre, gave moderate initial control, but lost effectiveness within 10 days (314).

Eriophyes pyri (pear leaf blister mite)—Malathion not effective (160).

Vasates atlantazalea (azalea mite)—Malathion gave excellent control (437).

Vasates cornutus (peach silver mite)—Malathion was ineffective for control of this mite; treated plots had more mites than controls (29).

Vasates fockeui (plum nursery mite)—Malathion not effective (31).

Vasates lycopersici (tomato russet mite)—Malathion has been reported to be: very effective as a dust and a spray (437); nearly as effective as sulfur but inferior to parathion (592); and as satisfactory as but less effective than parathion (12). Malathion gave the largest increase in crop yields (61), even though other materials gave better mite control (62).

Phyllocoptruta oleivorus (citrus rust mite)—Malathion not effective (581).

2. Insecta

a. Collembola. *Bourletiella hortensis* (garden spring tail)—Malathion dust, 1 lb. per acre, gave complete control (572).

Smynthurus viridis (lucerne flea)—Malathion gives spectacularly good control at 0.5 oz. per acre (607,609), some 400,000 acres being treated in 1956. Current recommendation is 1 oz. per acre (304).

b. Orthoptera. *Melanoplus mexicanus mexicanus; M. femur-rubrum* (grasshoppers)—On alfalfa, malathion at 0.625 lb. per acre gave moderate control of nymphs (410).

c. Thysanoptera. *Dendrothrips ornatus*—Malathion, 1.5 lb. per 100 gal., gave good initial control with some build-up of the population 3 weeks later (529).

Thrips tabaci (onion thrips)—In some centers onion thrips has developed resistance to a wide range of chlorinated insecticides, but not to organophosphorus insecticides (504).

An early report was that malathion gave fair control (218). In further tests malathion dust gave better initial control than parathion, methyl parathion, EPN, demeton, or DDT-BHC dusts, but was inferior to these and not very effective after 16 days (499,500). On cabbage, 1 lb. per acre failed to give control (645).

Sprays at 1.2 and 0.6 lb. per acre gave good control at 1 and 4 days, but control was usually poor at 8 days, although not always inferior to other organophosphorus insecticides (501–503). Medium gallonage sprays at 0.6 lb. of malathion per acre gave fair initial and subsequent control. The same amount applied at 4 gal. per acre gave good control and was still highly effective at 8 and 12 days after application when parathion had lost all residual effectiveness (504), suggesting that very low volume application of malathion might give spectacularly effective control of onion thrips.

Frankliniella spp. (and *occidentalis*)—Malathion, 0.31 lb. per acre, did not give adequate control of this thrips on cotton (133); at 0.75 lb. per acre good control of early infestations was obtained, but cotton yield was not increased (595).

Light infestations contaminating spinach were effectively controlled by 1 lb. per acre (255).

Frankliniella tritici; F. fusca; F. exigua (flower thrips)—One or two sprays of malathion, 0.625 lb. per 100 gal., gave very good control of *F. tritici* on blackberry fruits (590). On cotton, 0.25 lb. per 100 gal. gave good control at 2 days, but failed completely by 10 days (209). Four applications at weekly intervals of 0.31 lb. per acre gave poor control (132). Malathion was much inferior to dieldrin for impregnating the cheesecloth screening of rose houses for control of *F. tritici* and other thrips (315).

d. Hemiptera. *Magicicada septendecim* (periodical cicada)—Malathion not effective against adults (36,232,242).

Aphrophora parallela (pine spittle bug)—Complete control of nymphs by one application, 0.25 lb. per 100 gal. (325).

Philaenus leucophthalmus (meadow spittle bug)—On alfalfa one spray, 0.5 lb. per acre, gave excellent control of nymphs, with increased first cut yield, but was ineffective against adults, which damaged the second and third cuts (126). Malathion at 1 lb. per acre was not very effective, but 0.25 lb. of heptachlor (or 0.375 lb. of aldrin) + 0.625 lb. of malathion gave perfect control (33).

Circulifer tenullus (sugar beet leaf hopper)—In glasshouse tests, a 5% malathion dust gave fair initial control of adults, but the deposit was ineffective a week later (262).

Agallia constricta (leaf hopper)—On red clover, the numbers of this leaf hopper increased following one application of 1 lb. of malathion per 100 gal. (326).

Psallus seriatus (cotton flea hopper)—Malathion not very effective (133,656).

Macrosteles fascifrons (six-spotted leaf hopper)—On lettuce, malathion gave very rapid kill, being twice as fast as parathion, 4 times as demeton, and 8 times as DDT (575), but transmission of lettuce yellows was not satisfactorily prevented (576).

Empoasca decedens—Malathion not effective (140).

Empoasca fabae (potato leaf hopper)—Malathion dusts not effective (74). On alfalfa, 0.5 lb. per acre achieved immediate control, but this did not extend beyond 2 weeks (126). Very effective on potato and snap bean (653).

Empoasca solana (bean leaf hopper)—On beans, two applications, 1 week apart, of 0.6 lb. per 100 gal. gave rather poor control (539). In cotton fields, field application of 0.67 lb. per 100 gal. have poor control and was quite ineffective within 6 days. However, excellent results have been obtained with commercial applications by airplane of malathion for control of this pest on cotton (498).

Erythroneura variablis; E. elegantula (grape leaf hopper)—Malathion is used extensively for control of DDT-resistant hoppers; a 4% dust gave complete control 1 week after treatment, young nymphs appeared 10–14 days after treatment (561). A 4% dust at 20 lb. per acre usually gave 99–100% control of eggs, nymphs, and adults except during cool weather (46).

Erythroneura comes (grape leaf hopper)—Malathion, 0.25 and 0.37 lb. per 100 gal., gave good initial control, but reinfestation soon occurred (585).

Therioaphis maculata (spotted alfalfa aphid)—Malathion at 0.5–1.0 lb. per acre usually gives good control up to 15 days with less reinfestation at 3–5 weeks than other insecticides (58,336,487,495,569, 594). Exceptionally, 0.5 lb. per acre gave no reduction in aphid population 10 days after application (489). Populations about 4-fold resistant to parathion have appeared in California (568); against these strains malathion is sometimes effective, sometimes ineffective (570).

Control of fall aphid populations was readily achieved with one application of 1.0 lb. per acre; first cut yield was not affected, but crude protein and carotene content both improved (81).

Chromaphis juglandicola (walnut aphid)—One spray, 0.5 lb. per 100 gal., gave excellent control for 2 weeks; 0.75 lb. per 100 gal. gave perfect control for 1 month, trees becoming reinfested thereafter. Malathion was greatly inferior to schradan in over-all control (418,419).

Anuraphis helichrysi (leaf curl plum aphid)—Malathion gave good control when applied before leaves were curled (386,392).

Anuraphis roseus (rosy apple aphid)—Malathion at about 0.5 lb. per 100 gal. gave excellent control, even though the aphids live in rolled leaves and are thus protected (123). But severe infestations can occur even though the spray program contains malathion (211).

Aphis gossypii (cotton aphid)—On cotton, excellent control was obtained with one application of malathion at 0.4 or 0.75 lb. per acre (131,205,206). Infestations of this aphid, *A. rumicus,* and *M. persicae* on pears were effectively controlled by 0.5 lb. per 100 gal. (387).

Aphis pomi (apple aphid)—Malathion applied at 0.5 lb. per 100 gal. gave a complete kill (89); 4 lb. per acre gave effective control (475); at rates from 0.06 to 0.45 lb. per 100 gal., malathion gave high kill, but serious reinfestations occurred within 2 weeks (123.223). Two applications 6 weeks apart gave good control of *A. pomi* and *Amphorophora crataegi* on hawthorn; at 0.8 and 1.5 lb. per 100 gal., there was no reinfestation 9 weeks after treatment; at 0.375 lb. per 100 gal., reinfestation was very slight (528).

Aphis fabae (bean aphid)—One application, 1.4 lb. per acre, gave good control; at 0.75 lb. per acre medium volume sprays gave better control and higher yields than low volume application, but were much less effective than some systemics (616).

Aphis rumicis (black bean aphid)—Malathion sprays gave good control (149).

Brevicoryne brassicae (cabbage aphid)—A low volume spray of 0.8 lb. of malathion per acre gave excellent control for 7 days; dusts were also effective (623).

Hyalopterus pruni—In British Columbia, malathion was effective at 4 lb. per acre (475). In France, complete kill of this aphid was obtained with a single spray of malathion, 0.5 lb. per 100 gal. (89).

Capitophorus fragaefolii (strawberry aphid)—Malathion, 3.4 lb. per acre, gave excellent control, but cannot be used because strawberry mite, *Tarsonemus pallidus*, builds up after it has been applied (631).

Phorodon humuli (hop aphid)—Malathion, 1 and 2 lb. per acre, gave excellent control with minor reinfestation at 30–33 days (92,432).

Myzus cerasi (black cherry aphid)—One spray, 0.5 lb. per 100 gal., gave near perfect control (28).

Myzus persieae (green peach aphid)—Good control was obtained with 0.5 lb. per 100 gal. (475). On peach orchards in Washington, malathion has been widely and effectively used against this insect in the past, but in some orchards control has become difficult, suggesting that resistance has developed (30). Difficulty in controlling green peach aphid has also been experienced in Washington on potatoes, but in this case laboratory-reared colonies were easily controlled by malathion (349).

On egg plant, malathion, 0.5 lb./100 gal., gave complete control (159). For control of sporadic outbreaks on burley tobacco, malathion was much inferior to chlorthion and parathion (68), but on shade-grown tobacco weekly applications of malathion dust, 0.625

lb. per acre, gave excellent control, good grading index and higher yields than several other insecticides including parathion (236). Likewise, on shade-grown potatoes, malathion gave excellent control (604). On potatoes perfect control was obtained with sprays containing 0.25 lb. of chlordane + 0.125 lb. of malathion per acre (342).

Honey dew produced by this aphid (and *Aphis gossypii* and *A. rumicus*) may cause drop in young pears and speckling on mature pears. This damage is readily prevented by a petal fall spray of malathion, 0.5 lb. per 100 gal. (387).

Macrosiphum solanifolii (potato aphid)—Malathion gave good control on tomatoes (254,586). On potatoes perfect control was obtained with sprays containing 0.25 lb. of chlordane + 0.125 lb. of malathion per acre (342).

Macrosiphum pisi (pea aphid)—Malathion, the insecticide of choice for control of pea aphid, combines excellent control with freedom from taint and residues. A single application of 0.75–1.25 lb. pei acre has given excellent control on peas, lucerne, and red clover (59,146,147,187,307,326,654), with little build-up of population for 3 weeks (147,307) or before harvesting (187). Control was poor at 0.2 lb. per acre (127). On peas, adequate control was not obtained with 0.5 lb. per acre (130), although on alfalfa this dose gave adequate control for about 10 days (126) and on vetch very good control for 21 days, surpassing 0.25 lb. of parathion (488).

Eriosoma lanigerum (apple woolly aphid)—Use of DDT to control codling moth has resulted in tremendously increased woolly aphid infestations, usually controlled by lindane sprays applied early in the season. Malathion at 0.25–0.5 lb. per 100 gal. gave complete control (441). When trees are grown on aphid-susceptible stocks, there is both upward and downward aphid migration. When malathion sprays were applied at fairly long intervals, aphids in the aerial portion of the tree were controlled, but upward migration was unaffected. Downward movement built up rather rapidly, indicating a lack of residual effectiveness (393).

Psylli buxi (boxwood psyllid)—One application, 1.25 lb. per 100 gal., gave excellent control of adults (111), or fair control (529).

Psylla pyricola (pear psylla)—One application, 0.625 or 1.3 lb. per 100 gal., gave almost perfect control of nymphs. Although malathion was not nearly as ovicidal as parathion, over-all control was almost as good and not greatly affected by dose (435).

Psylla pyri (pear psylla)—In France, malathion, 0.8 lb. per 100 gal., gave very good control at 2 weeks, but was not sufficiently ovicidal to have long-term effectiveness (65).

Dysmicoccus obesus (loblolly mealybug)—A single application of malathion completely eliminated a heavy infestation (425).

Pseudococcus brevipes (pineapple mealybug)—Three sprays at monthly intervals, 2 lb. per acre, gave good control of mealybugs on aerial portions of the plant, but were ineffective against butt colonies (86).

Pseudococcus cuspidatae (taxus mealybug)—Complete control was obtained by a single application of malathion emulsifiable concentrate at 15 and 7.5 oz. of active insecticide per 100 gal. Less than complete control was obtained by a single application of malathion wettable powder at 8 oz. of active insecticide per 100 gal. (620).

Pseudococcus citri (citrus mealybug)—One application of malathion, 5, 2.5, or 1.25 lb. per 100 gal., gave complete control of young stages and good control of mature mealybugs (529). At 0.3 lb. per 100 gal., malathion was much inferior to parathion (340).

Pseudococcus maritimus (grape mealybug)—One application malathion, 0.25 lb. per 100 gal., gave a high initial kill, but failed to keep infestation under control (192). In a light infestation, 0.5 lb. per 100 gal. gave complete control (562).

Ferissiana virgata—Malathion gave complete control (275).

Eriococcus azaleae (azalea bark scale)—Complete control with malathion (528).

Coccus hesperidum (soft scale)—Outbreaks of soft scale have sometimes followed application of parathion for control of other pests. These outbreaks are probably due to the adverse effects of parathion on the parasite, *Metaphycus leuteolus*, which normally keeps populations of soft scale at a low level. A single spray of parathion, 0.5 lb. per 100 gal., gave 40% reduction in scales, single sprays of malathion, 0.125, 0.25, or 0.375 lb. per 100 gal., gave reductions of 78.3, 82.2, and 88.4%, respectively. When these amounts of malathion were combined with 0.125 lb. of parathion, the population reductions were 83.6, 91.5, and 94.4%. Increasing the amount of parathion in the mixture to 0.25 and 0.375 lb. per 100 gal. did not further reduce the population (174).

Lecanium pruniosum; L. corni; L. cerasorum (soft scales)—These scales on walnut are readily controlled by malathion (420,470).

Neolecanium cornuparum—Malathion gave perfect control (529).

Tourmeyella sp.—This scale on *Pinus virginanna* was completely controlled by one application of malathion, 3 lb. per acre (164).

Tourmeyella liriodentri—Malathion gave perfect control (529).

Tourmeyella numismaticum (pine tortoise scale)—Malathion was the most effective material per unit cost for use against crawlers of this scale (311).

Saissetia oleae (black scale)—Malathion alone is not effective against black scale on citrus. Sprays of 1% special light oil + 0.25 lb. of malathion per 100 gal. gave excellent control and did not accentuate water rot in navel oranges (119).

Diaspis carueli (juniper scale)—When junipers infested with this scale were sprayed with malathion, 1 lb. per 100 gal., fair control was obtained with one application, perfect control with two applications 15 days apart, or four applications about a week apart (69).

Parlatoria blanchardi (date scale)—Malathion not very effective (398).

Parlatoria olea (olive scale)—In extensive trials malathion was inferior to parathion (560).

Lepidosaphes beckii (purple scale)—Excellent control was obtained with three widely spaced sprays of 0.6 lb. per 100 gal. At 0.3 lb. per 100 gal., excellent control was obtained with emulsifiable concentrate, but wettable powder was inferior, allowing considerable increases in scale numbers (559).

Lepidosaphes ulmi (oyster shell scale)—One spray of malathion at 1.4 and 0.7 lb. per 100 gal. gave complete control of this single-brooded scale, if it was applied after most of the eggs had hatched; young from eggs hatched subsequently did not survive the residual action of malathion (528).

Phenacaspis pinifoliae (pine needle scale)—Infestations on *Pinus montana* were completely controlled by malathion applied once when hatching was partially over. The malathion-sprayed trees remained virtually free of second generation scales; the parathion-sprayed trees became heavily infested (528). On ponderosa pine a single application gave fair control; two sprays a month apart provided perfect control (476).

Aspidiotus ancylus (Putnam's scale)—Malathion not effective (176).

Aspidiotus forbesi (Forbes' scale)—On apples, malathion gave good control (244).

Aonidiella aurantii (red scale)—Malathion is effective for light and moderate infestations if applied in immediate postblossom period. Unlike parathion treatments, malathion treatments do not bring about a posttreatment increase in mites and scales (85). A spray containing 0.375 lb. of malathion + 1.0 or 1.8 gal. of special light oil was slightly less effective than a similar spray containing 0.19 lb. of parathion. Scale infestations in the plots treated with oil and malathion increased slightly each year; in the plot treated with oil and parathion they decreased slightly (120).

Fiorinia externa—Malathion-DDT mixtures gave excellent control of crawlers, but too many applications are required for their use to be practicable (129).

Aleyrodes spiaraeoides (iris white fly)—On potatoes in central Washington, malathion gave poor control (350).

Dialeurodes citri; D. citrifolii (citrus white fly)—Incidental results from experiments to control other citrus pests suggest that control of white flies would usually be satisfactory with three applications of malathion a year (559). Malathion gave good control of *D. citri* on *Gardenia* (589).

Trialeurodes vaporariorum (greenhouse white fly)—On outdoor beans in Hawaii, three applications malathion at weekly intervals gave excellent control up to 2.5 weeks beyond the last application. When applied at 2 week intervals, malathion was inferior to parathion (539).

Aleurocanthus woglumi (citrus black fly)—One spray of 0.35 lb. per 100 gal. killed all larvae and adults of *A. woglumi* on citrus in Pakistan (1).

Antestiopsis lineaticollis (coffee bug)—Application of DDT to control *A. lineaticollis* leads to outbreaks of the tingid *Habrochila ghesquiereri*. The latter can be controlled with parathion. Good control of both species can be obtained with a single spray containing 1 lb. of DDT + 0.5 lb. of malathion, this being superior to DDT or malathion alone at the same concentrations (186).

Aelia rostrata—Nymphs and adults of this pentatomid were easily killed by malathion deposits (6).

Murgantia histriomica (harlequin bug)—On eggplant a 4% malathion dust killed all bugs in 24 hours (76).

Anasa tristis (squash bug)—Malathion was inferior to parathion (259).

Lygus hesperus—A 5% malathion dust gave fair initial kill of adults, but was ineffective 1 week later (262); effective, but not better than DDT (84).

Lygus lineolaris (tarnished plant bug)—On alfalfa, a low volume spray, 0.625 lb. per acre, gave complete control of nymphs, no control of adults (410). This bug, which causes cat-facing on peaches, was controlled by a single petal fall spray containing 1.5 lb. of malathion per 100 gal. (64).

Blissus leucopterus (cinch bug)—Malathion not effective (210,490).

Nysius raphanus—Malathion sprays and dusts gave good control of this pest on vegetables, but the dead insects adhered tightly to the leaf surface, were difficult to shake or wash off, and proved very objectionable to shippers. This effect was not observed with other insecticides (625).

Gargraphia solani (eggplant lace wing)—Malathion was effective (76,653).

e. Coleoptera. *Agriotes lineatus*—Seed treatment with malathion dust was ineffective (87).

Carpophilus lugubris (sap beetles)—On sweet corn, 1.5 lb. of malathion + 2 gal. of oil per acre gave better control than either parathion or Diazinon (628).

Epilachna varivestis (Mexican bean beetle)—Although early trials (145) gave somewhat variable results, later trials have shown malathion to be outstandingly effective (75,218,654). Several applications at 5–15 day intervals of 1.5 lb. per acre gave almost complete control (71,73,149,150).

Epilachna vigintioctopunctata—One application of malathion at 0.75 lb. per acre gave adequate control (533).

Epicauta pestifera (margined blister beetle)—Malathion very effective (653).

Cotinus nitida (green June beetle)—Malathion was ineffective against larvae (128,155,531).

Popillia japonica (Japanese beetle)—Malathion sprays containing 1–3 lb. per 100 gal. gave complete control of adults on foliage (352). A later comment is that DDT and malathion are still the best chemicals for controlling this pest (471).

Macrodactylus pumilio—A 3% malathion dust gave an excellent kill of field-collected adults (2); deposits from sprays of 0.5 or 1 lb. per 100 gal. were ineffective (396).

Crioceris asparagi (asparagus beetle)—Malathion gave complete control (151).

Acalymma vittata; Diabrotica undecimpunctata howardi (cucumber beetles)—Good control was obtained with two applications of malathion, 0.34 lb. per acre (260).

Epitrix cucumeris (potato flea beetle)—Malathion is not effective (343).

Bruchus pisorum (pea weevil)—Malathion gave control equal to parathion but inferior to DDT (130,527).

Conotrachelus nenuphar (plum curculio)—Early reports indicated that malathion did not give effective control (506,556), although overwintered and first generation weevils were killed by malathion sprays at 0.5 and 1.0 lb. per 100 gal. (555,557) and malathion and parathion were equitoxic after 6 days despite the slower action of malathion throughout (97). More recent results indicate that malathion can give good control, but is inferior to parathion, and both are much less effective than dieldrin or parathion + dieldrin sprays (64, 457,558).

Anthonomus eugenii (pepper weevil)—Heavy, closely spaced applications of malathion failed to give control (175).

Anthonomus grandis (boll weevil)—Malathion not effective (133, 206,465,608). Eight applications within a month of a combined dust consisting of 2.5 lb. of malathion + 1 lb. of DDT per acre gave good control and a very large increase in seed cotton yield (112).

Endrin-resistant strains are more susceptible to malathion than nonresistant weevils (655).

Brachyrrhinus sulcatus (black vine weevil)—Malathion sprays, 1 lb. per 100 gal., gave better control than either DDT or parathion, but plants were heavily infested again in 2 weeks (438).

Pseudocneorhinus bifasciatus—4 and 2.5% dusts gave complete control; 1% dusts were ineffective (549).

Hypera postica (alfalfa weevil)—Malathion not very effective (33,59); 0.5 lb. per acre gave complete initial kill of larvae, but later control was poor (338). 6 oz. of heptachlor + 15 oz. of malathion in 25 gal. per acre is a standard and effective recommendation (466).

Hypera brunneipennis (Egyptian alfalfa weevil)—Against larvae on alfalfa foliage, 0.5 lb. of malathion per acre gave good initial control and at 8 days was superior to other materials tested; at 25 days malathion was less effective (496).

Hypera meles; H. nigrirostis (clover weevils)—Malathion, 1.25 lb. per acre, gave good control, but was inferior to dieldrin and endrin (51).

Hypera punctata (clover leaf weevil)—Malathion, 0.75 lb. per acre, gave a high initial kill, but the few survivors recovered and became adults; with heptachlor, endrin, and toxaphene there was considerable latent toxicity, many of the initial survivors remaining moribund and failing to become adult (565).

Hylastinus obscurus (clover root borer)—Poor control at 5 lb. per acre (649).

Listroderus obliquus (vegetable weevil)—Malathion dust, 1.2 lb. per acre, gave a measure of control (52).

Scolytus multistriatus (smaller European elm bark beetle)—Residues from 0.5 and 1.0% malathion sprays were ineffective against this beetle (152); emergence was not prevented when logs were sprayed with 0.5 and 1.0% emulsions (153).

f. Lepidoptera There are a number of lepidopterous pests which spend all or most of their larval period feeding within plant tissue. Control of these pests depends very largely upon killing eggs before the grubs hatch or upon killing newly hatched larvae as they enter the plant. Malathion, which lacks pronounced ovicidal and systemic effects, is not very effective against such insects. Pests in this category against which malathion has been found ineffective or inferior to other insecticides, notably parathion, include codling moth, *Carpocapsa pomonella* (222,245); European pine shoot moth, *Rhyacionia buoliana* (267,424); grape berry moth, *Polychrosis viteana* (116,583); corn borer, *Pyrausta nubilalis* (113–115,218,480); pickle worm, *Diaphania nitidalis* (166); currant clear wing, *Ramosia tupliformis* (584); peach tree borer, *Sanninoidea exitiosa* (548); corn earworm, *Heliothis zea,* in maize (268) and sweet corn (102,173); and the unspotted tentiform leaf miner, *Callisto geminatella* (292). As an apparent exception to this generalization, short interval sprays of malathion proved very effective against the cranberry fruit worm, *Mineola vaccinii,* equalling methoxychlor and surpassing DDT, TDE, lindane, and parathion. However, with this pest larvae do not immediately enter the berries and later pass from cluster to cluster (296).

Pieris rapae (imported cabbage worm)—Four fortnightly applications of malathion dust at 1.5 lb. per acre gave moderate control, but were inferior to DDT; wettable powder was less effective than dust (251). Malathion gave satisfactory control of cabbage worm resistant to chlorinated hydrocarbon insecticides (380).

Earias insulana—Spraying of bolls with 1.5 lb. of malathion per 100 gal. caused complete kill of larvae in 72 hours. In field tests malathion gave poor results (467).

Diparopsis castanea (red boll worm)—Repeated application of malathion gave good control (381).

Heliothis zea (corn earworm; boll worm; tomato fruit worm)—Two applications at 10 day intervals failed to give good control on sweet corn where infestation occurs during silking and later the larvae are protected (268); on beans, five applications of malathion at 1.5 lb. per acre (149) or four applications at 1 lb. per acre (150) gave satisfactory control; on tomatoes, malathion dusts and sprays gave poor control (634); on sorghum, one application of 1 lb. per acre gave fair control, but was inferior to both phosdrin and DDT (80,141). On cotton, 2.5 lb. of malathion + 1 lb. of DDT dust per acre gave good control (112), although malathion alone has been reported to be ineffective (608).

Marcua testulalis (bean pod borer)—Malathion gave commercial control (539).

Prodenia litura—In laboratory tests, malathion at 0.75 lb. per 100 gal. gave complete kill (228).

Trichoplusia ni (cabbage looper)—Malathion usually gives good control, but is sometimes ineffective (252,622,630,653). One application of malathion-perthane spray at the rate of 1 lb. of each material per acre gave outstandingly good control, although neither malathion at 1 or 2 lb. per acre nor perthane at 1 or 1.75 lb. per acre gave satisfactory control (56).

On cotton in Texas, six or eight sprays of 2.5 lb. of malathion + 1 lb. of DDT per acre gave perfect (624) or very good control (458).

Alabama argillacea (cotton leaf worm)—Malathion is widely used and gives excellent control (4,131,205,299,429,608).

Acontia dacia (cotton leaf worm)—Malathion at 0.25–0.5 lb. per acre gives excellent control (205,372,397).

Xylomyges curialis (citrus cutworm)—Malathion at 2.5 lb. per acre gave good control, but was not as effective as other insecticides (40).

Agrotis ypsilon; Pseudoaletia unipunctata; Peridroma margaritosa (cutworms and armyworms)—Malathion gave very poor control (488). Malathion was completely ineffective against *P. margaritosa* (447).

Hemerocampa vetusta (western tussock moth)—Malathion did not give effective control of this insect on citrus (41).

Carpocapsa pomonella (codling moth)—Malathion has usually been found ineffective or inferior to other insecticides against this pest (7,222,245). However, a grower program starting with lead arsenate at petal fall and then 12 days later, followed with four sprays in the next 6 weeks, of 0.75 lb. of DDT + 0.5 lb. of malathion per 100 gal. and with two final sprays of malathion, gave outstandingly good control of codling moth and of other orchard pests (243). Against DDT_r codling moth in South Australia, malathion, 0.5 lb. per 100 gal., at fortnightly intervals, gave excellent control (375, 553,554).

Cydia molesta (Oriental fruit moth)—Malathion was not as effective as other organophosphorus insecticides (64,599).

Cydia leucostoma (flush worm)—On tea in India, malathion sprays at 0.125 lb. per acre applied twice with an interval of 8 days gave some control, equalling DDT (491).

Cydia funebrana—Malathion was ineffective (88).

Rhopobota naevana (holly bud moth)—Malathion gave excellent control (580).

Ancylis comptana fragariae (strawberry leaf roller)—Malathion gave reasonable control, but was not better than parathion or TDE (277).

Spilonota ocellana—Malathion gave good control (459).

Argyrotaenia velutinana (red-banded leaf roller)—Malathion gave good control (456).

Clepsis peritana (garden tortrix)—Malathion less effective than other insecticides (39).

Pammene rhodiella—Malathion did not give good control (603).

Tortrix citrana (orange tortrix)—Malathion is as effective as parathion (388,391).

Anarsia lineatella (peach twig borer)—Malathion effectively prevented twig infestation (579).

Pectinophora gossypiella (pink bollworm)—Malathion less effective than other insecticides (70,637).

Segasta basquella (red-necked peanut worm)—Malathion not effective (35).

Plutella maculipennis (diamond back moth)—Malathion 0.28% at weekly intervals gave good control of a strain possibly resistant to DDT (273).

Argyresthia sp. (arborvitae leaf miner)—Malathion, 1 lb. per 100 gal., gave a very high kill (383).

Thyridopteryx ephemeraeformis (bagworm)—Malathion gave excellent control, killing most of the full-grown larvae, even though feeding had stopped (439).

g. Diptera. *Drosophila melanogaster* (vinegar fly)—Drosophila is often a severe pest at canneries, oviposition on ripe tomatoes in field and factory causing contamination in the canned product. Breeding in piles of cull fruit was effectively controlled by a single application of granulated 15% malathion (157).

Bait sprays containing 0.1% malathion in molasses were not very effective (401), but stronger bait containing 2.5 lb. of malathion and 8 lb. of sugar in 16 gal. of water per acre markedly reduced the population on the day of application and remained effective for a few days (421). Oviposition was materially reduced when a 0.55% malathion spray was applied to baskets of picked tomatoes (57).

In California these flies are increasing in importance as pests of figs, tomatoes, and seedless grapes. Laboratory evaluation of insecticides is being attempted. Among sprays containing 1% toxicant, malathion gave rapid knockdown of Drosophila, equalling Diazinon and surpassing lindane and parathion. Residues of malathion freshly deposited on glass gave more rapid knockdown than equal deposits of Diazinon, lindane, and parathion. When deposits were continuously exposed to an air current in the laboratory, only malathion, parathion, EPN, dieldrin, and DDT were effective after 1 week. malathion deposits giving the most rapid knockdown. Malathion deposits remained highly effective for another week and were still insecticidal after 5 weeks of weathering. At no time was the malathion residue inferior to the parathion residue (169). Other laboratory tests have confirmed that malathion has a high toxicity to Drosophila and that deposits are relatively persistent (156).

Scaptomyza graminum—This drosophilid was the dominant species in the maggot complex infesting bud and expanding leaves of spinach. Malathion at 0.75 and 1 lb. per acre did not give effective control (237).

Psila rosae (carrot rust fly)—A preplanting soil treatment of 6 lb. of malathion per acre was ineffective (522).

Dacus oleae (olive fly)—Three applications of 0.1 or 0.2% (521) or one application of 0.5% malathion did not give control (411). Three applications of 0.3% malathion emulsion gave considerable clean fruit, but parathion and Diazinon were superior (94). Similar results were obtained with malathion sprays at the very low concentrations of 0.06 and 0.03% (137). Timing of spray applications is very important, for malathion (unlike parathion) does not kill larvae within the fruit (93). Low volume sprays of about $^1/_{10}$ normal gallonage containing 30 lb. of malathion per 100 gal., three applications about a month apart, gave almost complete control when almost 82% of control fruit were infested (412).

Dacus cucurbitae (melon fly)—When malathion sprays of 0.5 lb. per 100 gal. and bait sprays containing 0.5 lb. of malathion + 1 lb. of yeast hydrolyzate were compared by application to isolated plants, bait sprays gave a higher kill of melon fly adults. The period of effectiveness of both baited and nonbaited sprays was short, about 1–2 days. When the sprays were field tested for control of melon flies attacking cucumbers and cantaloupes, there were no differences in fly population or fruit infestation between baited and nonbaited plots (444).

Vegetation surrounding the crop usually harbored more flies than the crop; serious crop infestation was prevented by spraying this border vegetation every 2–3 weeks with malathion. When spraying was discontinued, the fly population increased markedly (443).

Dacus orientalis (Oriental fruit fly)—Outstandingly good control was obtained with bait sprays comprising 1 lb. of malathion + 1 lb. of yeast hydrolyzate or partially hydrolyzed yeast protein in 3–150 gal. of water per acre. Good control was obtained with full cover sprays of malathion at 5–6 lb. per acre, but this insecticide was rarely effective for more than 7 days when applied at less than 3 lb. per acre. Apparently malathion is highly toxic to fruit flies when ingested, as weathered deposits, which failed to kill flies confined to them for 24 hours, could be made effective by application of protein hydrolyzate over them (566,567).

Ceratitis capitata (Mediterranean fruit fly)—A bait spray comprising 1 lb. of protein hydrolyzate or a quart of sauce base and 2–3 lb. of 25% malathion wettable powder per gal. per acre is being used extensively in the campaign to eradicate Mediterranean fruit fly from Florida. The spray is applied at intervals of 10–14 days, depending on the frequency and intensity of rain (23,534).

A bait spray containing 1% malathion in 10% sugar solution applied two or three times at intervals of about 10 days totally prevented infestation of apricots in Cyprus (215).

A bait spray containing 2 lb. of malathion + 50 lb. of molasses per 100 gal., applied at about fortnightly intervals, gave very good control of adult *C. capitata* and *Anastrepha mombinpraeoptens* in large areas, but was ineffective in areas smaller than 2.5 acres, owing to continuous immigration of flies (479). Six applications of a bait spray containing 1 lb. of malathion + 30 lb. of sugar per 100 gal. did not give as good a result as a similar spray containing dieldrin (478).

A 0.3% malathion spray containing sugar remained effective for about a fortnight and was greatly superior to other materials (468). A bait spray containing 0.5% malathion and 5% sugar applied at irregular intervals at 5 gal. per acre gave rather poor control compared with a dieldrin spray; inclusion of a partly hydrolyzed protein material increased the attractiveness of the malathion sugar bait almost 5 times (214). Sugar or protein hydrolyzate bait sprays were only partially effective in Algeria (191).

Rhagoletis pomonella (apple maggot)—Malathion failed to give control (136).

Rhagoletis cingulata (cherry fruit fly)—Malathion was not effective for control (117,486). In laboratory trials malathion had about $^1/_8$ the toxicity of parathion against adults (193) and did not give effective control of maggots within the fruit (195).

Rhagoletis completa (walnut husk fly)—Malathion, either as a cover spray or plus protein hydrolyzate as bait spray, gave good control (47).

Monoarthropalpus buxi (boxwood leaf miner)—Malathion gave complete control at 1.5 lb. per 100 gal., poor control at 0.75 and 0.38 lb. (528).

Liriomyza sp.; *L. pusilla* (leaf miner)—Malathion was ineffective for control (629,644; cf. 303).

Hylemya antiqua (onion maggot)—Malathion, furrow sown with the seed at 2 lb. per acre, was completely ineffective (293).

Hylemya brassicae (cabbage maggot)—Malathion quite ineffective as a seed dressing even at 0.22 lb. 1000 foot row (571).

Hylemya cilicrura—Malathion, 1.5 lb. per acre, completely prevented infestation in spinach leaf clusters (612).

h. Hymenoptera. *Neodiprion lecontci* (red-headed saw fly)—Malathion was considerably more rapid in action than either DDT or methoxychlor. One spray of 1 lb. per 100 gal. gave complete kill; at 0.5 lb. per 100 gal. kill was 99.7% (324).

Athalia rosac (turnip saw fly)—A malathion spray at 1.0 and 0.5 lb. per acre gave good control (66).

Pristiphora erichsonii (larch saw fly)—In laboratory tests, malathion was more toxic than DDT and about equally effective with BHC (163).

Fcnusa pusilla (birch leaf miner)—First brood completely controlled by malathion (528).

C. STORED PRODUCTS PESTS

Malathion appears likely to be useful in control of stored products pests but information is still meagre.

1. Acarina

Very few adult grain mites, *Acarus siro*, penetrated through 12.5 mm. of flour treated with malathion at the very high dose of 4380 p.p.m. However, at this dose the protectant value of malathion was not superior to lindane or Neotran* (339). Residual deposits up to 200 mg. per square foot were virtually ineffective against *A. siro* and *Tyrophagus* sp. (400).

2. Insecta

Malathion is exceedingly toxic to adult *Oryzaephilus surinamensis;* highly toxic to adult *Tribolium castaneum*, *Calendra granaria*, and *Ephestia elutella;* but less effective against adult *Lasioderma serricorne* and *Rhizopertha dominica* and larvae of *E. elutella* and *Trogoderma granarium*. Adult *Stegobium paniceum* was little affected and *Ptinus tectus* virtually immune (462).

Malathion deposits were highly toxic to adults and larvae of the Indian meal moth, *Plodia interpunctella*, deposits from wettable powders being vastly more effective than deposits from emulsions.

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* Registered trademark Dow Chemical Co., Midland, Mich.

Aging deposits for 28 days before exposing the larvae did not affect the kill (313).

At 200 mg. per square foot malathion caused paralysis and death of larvae of *E. elutella* more rapidly than did deposits of 20 mg. of lindane or 80 mg. of DDT per square foot (21). Deposits of wettable powders on bricks soon lost toxicity to *E. elutella* larvae; possibly the alkalinity of the bricks brought about this deterioration (462).

No live rice weevils, *C. oryzae*, were found 6 months after infested maize was placed in cloth bags previously sprayed on the inside with 1% malathion; other insecticides were equally effective (541). Malathion dusts at 4 p.p.m. and above protected wheat for more than 3 months against the lesser grain borer, *R. dominica*, the granary weevil, *C. granaria*, and the rice weevil, *C. oryzae*. Even at 2 p.p.m. there was complete protection for at least 1 month (366). Later work has shown that 10 days exposure to grain treated 4 or 5 months earlier with 8 p.p.m. of malathion will result in a complete kill of adult *C. granaria* and *C. oryzae; R. dominica* was less susceptible, a complete kill not being obtained even immediately after treatment with 16 p.p.m. (234).

The concentration of malathion in parts per million, needed to suppress reproduction was: *O. surinamensis*, 0.5; *T. castaneum*, 0.5–1; *S. paniceum*, 1–2; *L. serricorne*, 4–8; *R. dominica*, *C. oryzae*, and *C. granaria*, 8; *P. tectus*, >8 (462). Wheat treated with 4 p.p.m. remained toxic to *Laemophloeus ferrugineus* for 12 months (19). But dipping the seeds of koa haole, *Leucaena glauca*, infested with *Araecerus levipennis*, in a 0.1% malathion emulsion did not prevent posttreatment feeding (537), although malathion applied at 8 p.p.m. to shelled maize and ears prevented reinfestation by the scolytid weevil, *Plagiocerus fiorii* (657). Encouraging results have also been obtained in tests with malathion wettable powder to minimize infestation of pyramids of bagged groundnuts by *Tribolium* and premises by *Trogoderma granarium* (25).

Talc was a less effective carrier than kaolin when dusts were admixed with wheat. With talc as the carrier more than 8 p.p.m. was needed to prevent breeding of *C. granaria*, *C. oryzae*, and *R. dominica*, while 2 p.p.m. prevented breeding of *O. surinamensis*. The corresponding values using the kaolin carrier were <4 and <0.5 p.p.m. It is thought that the differences may be due to the poor adhesion of talc dust to grain and insect (462).

In Canada, malathion may be used commercially for control of stored products pests, approval having been given to labels which advocate the use of 0.5% (w/w) malathion dust, mixed with grain at the rate of 1 lb. per 10 bushels (8 p.p.m. of malathion) for control of *L. ferrugineus*, *C. granaria*, *T. confusum*, *O. surinamensis*, *P. interpunctella*, and *Tyroglyphus;* and malathion has been approved for spraying empty granaries (17,462).

Laboratory tests have shown that the moisture content of grain has an important influence on effectiveness of malathion against *L. ferrugineus*. A complete kill was obtained at each concentration (2, 4, 8, 16 p.p.m.) when the wheat moisture content was 13.5% and tests were made either 2 or 5 months after treatment. With higher moisture content (18.0%) only 16 p.p.m. gave complete protection and then only at 2 months. Subsequent infestation was closely related to mortalities of the test insects; but even when test insect mortalities were high, some oviposition and development recurred (615).

Adult *S. oryzae* moved freely from untreated grain into malathion-treated grain, indicating that malathion is not repellent. This may be of considerable practical importance if treatment is uneven, for treated grain may trap insects that move out of the untreated portions (615).

An unusual infestation of corn cob litter in a brooder house with the lesser meal worm, *Alphitobius diaperinus,* was readily controlled by a malathion spray applied at 1.25 lb. per 1000 square feet; DDT was even more effective (257).

Malathion may prove useful for control of dermestid larvae, which are notoriously insensitive to contact insecticides. Malathion in white oil gave rapid knockdown of larvae of the khapra beetle, *Trogoderma granarium,* but death occurred only after 3–4 weeks continuous exposure (462). The fourth instar larvae had an LD_{95} of 3.6 μg. of malathion per larvae, compared with more than 100 μg. for many insecticides (chlordane, DDT, TDE, dieldrin, methoxychlor, toxaphene, etc.), 50 μg. for lindane, and 29 μg. for allethrin. Only methyl parathion and parathion were more toxic than malathion, but mammalian toxicity precludes their use in most circumstances (367). In preliminary trials malathion at 4 p.p.m. on wheat appeared to control khapra beetle effectively (368). Other trials have shown that although large larvae are not controlled by 8 p.p.m., breeding is prevented by 0.5–1 p.p.m., suggesting that newly emerged adults, eggs, or young must be very susceptible (462).

Residual deposits of 100 mg. per square foot remained effective against *Trogoderma inclusum* for 3 months and against *Attagenus piceus* for 5 months; addition of piperonyl butoxide increased the period of residual toxicity (399). A spray containing 3% malathion in an odorless distillate gave complete control of larvae of black carpet beetle, *Attagenus piceus*, whereas other insecticides (excluding DDT) did not (422).

D. MISCELLANEOUS PESTS

Caloglyphus anomalus; Macrocheles sp.; Parasitus sp.—These mites infecting earthworm beds could not be controlled by malathion without also killing the earthworms (297).

Helix aspersa (European brown snail)—Malathion was not an effective toxicant in bran baits even with addition of metaldehyde (455).

Scutigerella immaculata (garden centipede)—Malathion less effective than parathion (294,295).

Blattella germanica—Complete control of a chlordane-resistant strain was obtained with one to three applications of a 2:1 mixture of Perthane and malathion (650). Sprays of 2% malathion gave good control up to 7 weeks (319), but more than one application was sometimes necessary (373).

Polistes exclamans—Malathion not very effective (289).

III. Effects on Pest Nematodes

Plants: Malathion at 4 g. or less per square foot applied as water emulsion had some therapeutic value when applied to soil in which *Meloidogyne incognata*-infested tomato plants were growing (582).

Animals: When hens were fed 100 p.p.m. of P^{32}-malathion in the diet, large numbers of dead round worms were eliminated in the droppings in the first few days (394). Chickens given heavy doses of malathion often voided the chicken round worm, *Ascarida galli*, in their droppings, but many more were found in the intestine upon necropsy (199).

These two observations suggest that it might be profitable to investigate the use of malathion as an antihelminthic in livestock.

IV. Effects on Beneficial Organisms

A. INSECTS

The effect upon beneficial insects is one factor that should always be considered when assessing the utility of an insecticide. Sometimes it is imperative that a crop be protected with insecticide, even though that insecticide is known to have adverse effects upon parasites and predators. But there is one group of beneficial insects, the bees, which must always be protected against the adverse effects of insecticides. However, because almost any material is likely to kill some bees, this protection cannot be absolute. Death of a major portion of the field force cannot be tolerated, however, even though it is probably true that the bee-keeper may be more affected by the time of insecticide application than by the number of bees killed; for, although a high kill of bees does not always hurt a colony substantially, loss of the foraging population for a short period during a heavy nectar flow may spell the difference between a good and a poor honey crop for the bee-keeper. Lieberman and his colleagues (361) consider that the limit of sanction has been reached when a field-applied insecticide kills 10% of the field force working the treated field. There is, of course, ample room for the use of judgment in particular cases, the important factor being the proportion of bloom within foraging distance that is treated at a particular time.

When field bees were caged with flowers that had been treated with a 1% malathion dust, all bees were dead within 5 hours; when bees were dusted directly with a 1% malathion dust, all were dead within 20 minutes. This was a more rapid action than a 1% parathion or a 2% BHC dust, both of which also caused complete kill (15). Laboratory tests showed that a 2% malathion dust was always considerably quicker acting than a 5% DDT dust and at the dosages used resulted in a complete kill of test bees, while DDT did not (42). When alfalfa was dusted with malathion at 1.6 lb. per acre all caged bees were killed; a spray at 1 lb. per acre killed 71% of caged bees. When bees were caged with sprayed foliage 1 day after spraying, 22% were killed, and at 3 days 15% (307). Application of 0.63 lb. per acre killed almost half the field force (361). An air-applied spray of 0.5 lb. per acre reduced the field population of bees to zero within a few minutes of application, but 6 hours later bees began to reappear in treated areas and by next morning bee activity was back to normal. There was little if any effect on the colony, probably because many of the bees were working in areas other than those treated. A similar result was obtained with 0.75 lb. per acre and, additionally, there was a complete kill of bees placed on the foliage soon after treatment, and 6.5 hours after treatment. Two hours later the foliage was not toxic to caged bees (13). Although malathion is moderately toxic to bees, it appears to be less toxic than parathion and has been used extensively on blooming alfalfa without serious loss of bees. If all fields in the area were treated at the same time, bee loss might be heavy (13).

A later comment is that malathion is inconsistent in toxicity to bees. Many thousand acres of flowering alfalfa have been treated with malathion without serious loss to colonies, but occasionally heavy losses do occur, particularly at extremely high temperatures. Use of malathion around bees is therefore questioned (14). Perhaps further investigation is warranted in view of the claim that application of malathion to flowering borage rendered the nectar toxic for several days (407).

In the campaign to eradicate Mediterranean fruit flies malathion bait sprays were applied at 10 day intervals over very large areas at 0.5 lb. of malathion per acre. This widespread use of malathion has not affected seasonal changes in weight of honey bee colonies (646).

The effects of malathion on beneficial insects other than honey bees has not been studied extensively. A standard laboratory bell jar test with a wide range of insecticides showed that the organophosphorus insecticides parathion, malathion, Chlorthion, and Diazinon are far more toxic to the convergent lady beetle, *Hippodamia convergens*, and the spotted lady beetle, *Coleomegilla maculata*, than are the chlorinated hydrocarbons DDT, Perthane, Strobane,* heptachlor, toxaphene, endrin, and dieldrin (261). This susceptibility has been confirmed in the field. In alfalfa fields malathion at 0.71 lb. per acre caused a large mortality of larvae and adults of *Hippodamia* sp., an important ladybird predator of the alfalfa aphid, *Therioaphis maculata*. Spraying reduced the aphid population from 52 to 12 aphids per stem, but 11 days after spraying there were 12 times as many aphids on sprayed plots as on the controls. Aphids on sprayed plots had not increased, those on unsprayed plots had been almost wiped out by predators (596). With predatious Hemiptera, adult *Orius* and *Geocoris* were little affected, adult *Nabis* were seriously reduced, larval *Orius* were wiped out, the larvae of *Geocoris* and *Nabis* were almost unaffected (84,596).

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* Registered trademark B. F. Goodrich Chemical Co., Cleveland, Ohio.

Treatment of alfalfa with 0.5 lb. of malathion per acre did not greatly reduce the total arthropod population, although after spraying there were fewer predators and parasites than aphids, where previously the enemy complex had outnumbered aphids (183).

Attempts to find insecticides effective against alfalfa aphid but relatively nondamaging to parasites and predators are being made. Tests with three Hymenopterous parasites, *Praon politans*, *Trioxys utilis*, and *Aphelenius semiflavus*, showed that malathion deposits were more toxic than those of a large number of other insecticides. Further, at high doses parasite pupae may be killed, even though they are within the mummified aphids (48). For such work, a selectivity index has been suggested based on the difference between the doses required to kill 95% of the pest species and 5% of the beneficial animals. With alfalfa aphid and two ladybird species, adult *Hippodamia quinquesignata*, and larval *H. convergens*, the selectivity index is often surprisingly high, indicating that many insecticides provide good differential toxicity in favor of the beneficial organisms. Malathion and parathion were the least selective of the materials tested, their selectivity indices being negative (48).

To the predatious mite, *Typhlodromus fallacis*, malathion was as toxic as parathion, and these were the most toxic of the 25 insecticides and fungicides tested, which included dementon, schradan, DMC, DDT, lindane, heptachlor, dieldrin, chlorobenzilate, aramite, sulfur, captan, and bordeaux (507).

In Nova Scotia apple orchards, malathion sprays had a drastic effect upon the eleven predators studied. Nine—three predacious mites, four mirids, one acanthocorid, and one thrips—were practically eliminated, while two—the predatious mite, *Mediolata novae-scotiae*, and the thrips, *Leptothrips mali*—were reduced in numbers. Comparison with the other acaricides, insecticides, and fungicides indicates that organophosphorus insecticides present the greatest hazards to beneficial insects (8,79,384,475).

Occasionally it is necessary, or thought to be necessary, to control species usually considered to be beneficial. Tiger beetles, *Cicindela* *haemorrhagica,* thought to be predators of the alkali bee, *Nomia melanderi,* were unaffected when their nesting sites were just wet with a spray containing 0.625 lb. of malathion per 100 gal. At double the dose the kill was 44% (194).

B. EARTHWORMS

Organophosphorus insecticides are more toxic than chlorinated insecticides to English red worms, *Eisenia foetida,* the LD_{50} for malathion being 1.5 lb. per acre compared with about 40 lb. for aldrin and heptachlor (288).

C. BENEFICIAL FUNGI

Laboratory tests with insecticides against the vegetative stage of five species of *Entomophthora,* which attack and in part control spotted alfalfa aphid, showed that malathion was toxic to four species. Other insecticides were also toxic; trithion was toxic to all species, while parathion and DDT were the most innocuous. The dominant pathogen *E. exitialis* was susceptible to all the insecticides, but in practice few insecticide applications are made when fungi are active. Insecticides do not affect viability of spores of *E. virulenta,* and if other species are similar it is likely that present insecticidal programs do not have adverse effects upon survival of these beneficial fungi (239).

V. Effects on Wildlife

Widespread use of pesticides has created problems through unintentional poisoning of wildlife. A survey and extensive review of these problems states that there has been no report of wildlife loss from malathion and suggests that malathion might substitute for some of the highly hazardous insecticides (520).

However, field observations following aerial application of malathion at from 0.2 to 0.75 lb. per acre indicated extensive mortality of killifish, *Cyprinodon, Fundulus, Gambusia,* and *Mollienesia* (125). In field trials with *Fundulus,* malathion at 0.5 lb. per acre killed a third of the test fish, and another third were affected but not killed (125).

In laboratory trials, malathion at 0.05 p.p.m., i.e., approximately the minimum lethal dose for field control of mosquito larvae, killed

40% of the surface-feeding minnow, *Gambusia affinis* (358). At 0.1 p.p.m. malathion was decidedly toxic to salmon fingerlings, but the next lowest concentration, 0.032 p.p.m., killed no fish in 6 days. Intervals of up to 6 (461) or 14 (18) days between preparing the malathion dilutions and introducing the test fish did not affect toxicity. Three p.p.m. killed 60% of carp (266); 5 p.p.m. was lethal to fingerling bluegills, goldfish, rainbow trout, sunfish, and yellow perch; sea lamprey larvae were unaffected (33a,648). Malathion was practically nontoxic to fathead minnows; the 24 hour LD_{50} was 25 p.p.m. (271); 95% survived a dose of 21 lb. per acre (278).

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